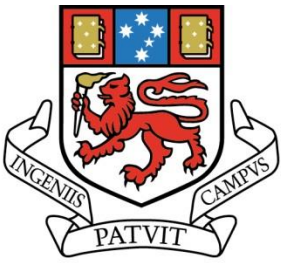

UNIVERSITY
OF TASMANIA

The influence of late-life university education on age-related cognitive decline and cognitive reserve:

The Tasmanian Healthy Brain Project

By Megan Elizabeth Thow

BArts (Hons.)

School of Psychology

Submitted in fulfilment of the requirements for the Degree of Doctor of Philosophy

University of Tasmania, June 2015

Declaration of Originality

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in this thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

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Located in Appendix D.
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Paper 2, Relationship between education and age-related cognitive decline: A review of recent research
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Paper 3, Sending your Grandparents to university increases cognitive reserve: The Tasmanian Healthy Brain Project
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Paper 5, Does *APOE* allelic variation modify responsiveness to a tertiary education intervention designed to enhance cognitive reserve: The Tasmanian Healthy Brain Project
Located in chapter 6
Candidate was the primary author (60%) and with author 2 contributed to the idea, its formalisation and development, refinement and presentation (30%). Author 3 (5%) and 4 (5%) assisted with refinement and presentation.

Paper 6, Does *BDNF* Val66Met polymorphism modify responsiveness to a tertiary education intervention designed to enhance cognitive reserve: The Tasmanian Healthy Brain Project
Located in chapter 7
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Paper 7, *APOE* and *BDNF* polymorphisms do not interact to modify cognitive performance over a 4 year period: The Tasmanian Healthy Brain Project.
Located in chapter 8
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We the undersigned agree with the above stated "proportion of work undertaken" for each of the above published (or submitted) peer-reviewed manuscripts contributing to this thesis:

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Statement of Unique Contribution

This PhD was commenced after the first year of data collection had been completed, consequently the candidate did not contribute to the original design of the study, or contribute any additional measures to the design. However, a number of unique contributions were made in the form of papers not included in the original project design developed by the Chief Investigators of the study, specifically Paper 1 (presented in Appendix D) and Paper 2 (presented in Chapter 4). In addition, the design of all statistical analysis conducted in all chapters of the thesis was a unique contribution made by the candidate.

The initial analysis plan formulated by the THBP Chief Investigators was to use mixed group repeated measures analysis of variance to examine the effect of participation in the THBP, with participants grouped according to study load undertaken, and performance on repeated neuropsychological measures being assessed over time (Summers et al., 2013). The second analysis planned by the THBP Chief Investigators was to identify the factors associated with decreased age-related cognitive decline using multiple regression analyses (Summers et al., 2013). The third aim initially conceptualised by the THBP Chief Investigators was to identify whether genetic markers acted as a mediator of the relationship between educational enhancement and age-related cognitive decline, using a mixed-group repeated measures MANOVA (Summers et al., 2013).

The candidate reconceptualised her own research aims and subsequent analyses, which were unique from this initial conceptualisation of the THBP. The analyses planned and carried out by the candidate made full use of the limited amount of data available at this early stage of the THBP, because it was not necessary for missing data to be excluded. Firstly, growth mixture modelling was used to determine whether participation in the THBP had increased

cognitive reserve. The latent groups identified in this analysis (based on whether cognitive reserve remained stable, increased, or decreased) were then used as the grouping variable in all subsequent analysis. The candidate was then able to use multiple group latent growth curve modelling to examine whether those participants in the THBP who had shown an increase in cognitive reserve displayed a significant change in four specific neuropsychological functions, relative to controls for whom cognitive reserve had remained stable. Similarly, multiple group latent growth curve modelling was used to examine whether specific genetic variations contributed to different patterns of age-related cognitive change.

Statement of Ethical Conduct

The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator, and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University.

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Lenehan, M. E., Summers, M. J., Saunders, N. L., Summers, J. J., Ward, D.D., Ritchie, K., & Vickers, J. C. (2015). Sending your Grandparents to university increases cognitive reserve: the Tasmanian Healthy Brain Project. *Neuropsychology, accepted pending minor revision.*

Abstract

Background: A strong link between education and cognitive performance suggests that a period of education in later-life could reduce age-related cognitive decline (ARCD) and risk for Alzheimer's disease (AD). The aim of this thesis was to examine the effect of late-life education on cognitive reserve (CR), cognitive functioning; and the potential influence of genetic factors on any relationship.

Method: A sample of 459 participants aged 50-79 years (M = 60.24, SD = 6.75) enrolled in the first four years of the THBP, provided salivary samples for genetic analysis and completed comprehensive annual cognitive assessments. Within this sample, an intervention group (n = 359) who undertook a minimum of 12 months part-time university level education were compared with a control reference group (n = 100).

Results: Growth Mixture Modelling (GMM) revealed that while 92.5% of the intervention group displayed an increase in CR, only 55.6% of the control group displayed an increase. Further, the intervention group displayed a significant increase in language processing capacity but no significant change in episodic memory, working memory or executive function. There was no influence of genetic factors (*APOE* ε4 or *BDNF* Val66Met) on cognitive function over time or on intervention response.

Conclusions: Attending university improved CR and triggered a commensurate improvement in crystallised cognitive function (language processing capacity) but not fluid cognitive functions (episodic memory, working memory or executive function). These results indicate that encouraging mental activity in later-life may be a viable means to reduce ARCD and potentially delay the onset of AD.

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A big thankyou to my supervisor Mat – I am so grateful for all of the support you have given me during my candidature. I have thoroughly enjoyed working together on this project and learning from your huge wealth of knowledge and expertise. From your office at UTAS and then all the way from your office with a view at USC in sunny Queensland, supervision hour has been something I always looked forward to and left feeling uplifted and inspired.

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Chapter 1

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder that results in a gradual progressive decline in higher order cortical functions and results in eventual death. AD is the most common type of dementia, accounting for approximately 50% of dementia cases (Access Economics, 2009). It is recognised by the Australian Government as a National health priority (Butler, 2012) and was recently revealed to be the second biggest causes of death among Australian's after heart disease (Australian Bureau of Statistics, 2015). Consequently, it is of high importance to devise ways of delaying the onset of dementia and understanding the mechanisms that contribute to individual differences in rates of age-associated cognitive. If research identifies those factors leading to greater cognitive function in later-life, then interventions could be applied to maximise cognitive function and thereby delay or prevent the onset of this insidious disease.

One non-pharmacological approach to reducing the risk of rapid age-related cognitive decline and AD is to increase cognitive reserve (CR). The concept of CR suggests that individuals vary in their capacity to utilise pre-existing cognitive strategies or enlist alternate brain efficiently when under the duress of brain pathology (Stern, 2009; Tucker & Stern, 2011). Education is thought to be a key contributor to CR (Stern, 2009). Therefore, enhancing an individual's level of CR through education has the potential to preserve normal cognitive function for a longer period of time in the presence of the neuropathological changes in the brain associated with age. However, despite the strong link between early-life education and cognitive function in later-life, the potential benefit of an education based intervention in later-life has not been directly examined.

Dementia in Australia

Australia, like many economically developed nations, has an aging population. With an unprecedented increase in life expectancy occurring over the last century, as well as large cohorts of post-WWII 'baby boomers' now entering the over 65 age group, Australia's population aged 65-84 years is expected to reach up to 6 million in 2056. This more than doubles the 2.4 million people in this age cohort in 2007 (Australian Bureau of Statistics, 2009). Similarly, the number of Australian's aged 85 years was 344,000 in 2007 and is expected to increase to 1.7 million in 2056 (Australian Bureau of Statistics, 2009). There is a subsequent rise predicted for incidence of dementia.

Dementia: Prevalence Estimates, Projections and Costs

In 2009, Alzheimer's Australia commissioned Deloitte Access Economics to provide prevalence estimates and projections for Australia. The report showed that dementia constitutes the leading cause of disability in Australian adults aged over 65 years (Access Economics, 2009). In 2009 there were an estimated 266,514 people living with dementia in Australia (Access Economics, 2009). By 2050, this figure is predicted to rise to nearly 950,000 representing a growth of about 254% over a 39 year period (Access Economics, 2009). By mid-century, there will be over 1.13million Australians living with dementia (Access Economics, 2009). Data reported by Anstey et al. (2010), which was drawn from a pooled dataset of Australian studies and two national surveys, supports such projections and suggests they may even be conservative.

Dementia constitutes a substantial economic burden in Australia. In 2003, the cost of Alzheimer's disease, which is just one of the multiple causes of dementia, was estimated to be approximately $3.2 billion (Access Economics, 2004). By 2060 this figure is projected to increase to up to $83 billion Australian dollars, which equates to 11% of spending in the health and residential care sector (Access Economics, 2004).

Delaying the Onset of AD

It has been projected that if the onset of Alzheimer's disease could be delayed, even by just 5 months then the incidence of dementia could be substantially reduced, saving billions of dollars per year (Access Economics, 2004). Dementia also exerts a huge impact on quality of life for those with dementia and their families. For these reasons, it is of considerable importance to devise ways to delay the onset of dementia and understand the mechanisms that contribute to individual differences in rates of age-associated cognitive decline. If research identifies factors that enhance cognitive function in later-life, then interventions could be applied to help prevent or decrease the severity of age-associated cognitive decline. If people are performing at a higher level of cognitive function to begin with, it stands to reason that they will be able to withstand a greater amount of cognitive decline before functional or clinical impairment is apparent.

Age-related Cognitive Decline

Subjective experiences of memory and other cognitive problems are a common complaint as people get older (Fritsch, McClendon, Wallendal, Hyde, & Larsen, 2014). Most people will

experience greater cognitive frailty with age and this is a normal (non-pathological) and expected part of the aging process (Hedden & Gabrieli, 2004). The typical cognitive aging profile is well described in the literature and will be described below.

The Prototype of Age-Related Cognitive Decline

Extensive research on the effect of age on cognition has revealed three distinct patterns of cognitive change. These are: (1) cognitive decline occurring across the life course; (2) late-life cognitive decline; and, (3) relative cognitive stability throughout life. These different patterns suggest that although aging has an overarching impact on cognition, the aging process effects the cognitive functions differentially (Hedden & Gabrieli, 2004).

Late-life declines and relative stability.

Up until 50 - 60 years of age, there is an increase in performance on measures involving well-practiced tasks or tasks that involve accumulated knowledge acquired earlier in one's life, such as knowledge of vocabulary, semantic memory and general information (Hedden & Gabrieli, 2004; Salthouse, 2010b). These functions remain relatively stable until very late-life, when a small decline is evident (Hedden & Gabrieli, 2004; Salthouse, 2010b). This is observed consistently across both cross-sectional and longitudinal data sets (see Figure 1) (Hedden & Gabrieli, 2004). Based on the limited research available, autobiographical memory and procedural memory also appear to be preserved throughout the lifespan (Hedden & Gabrieli, 2004).

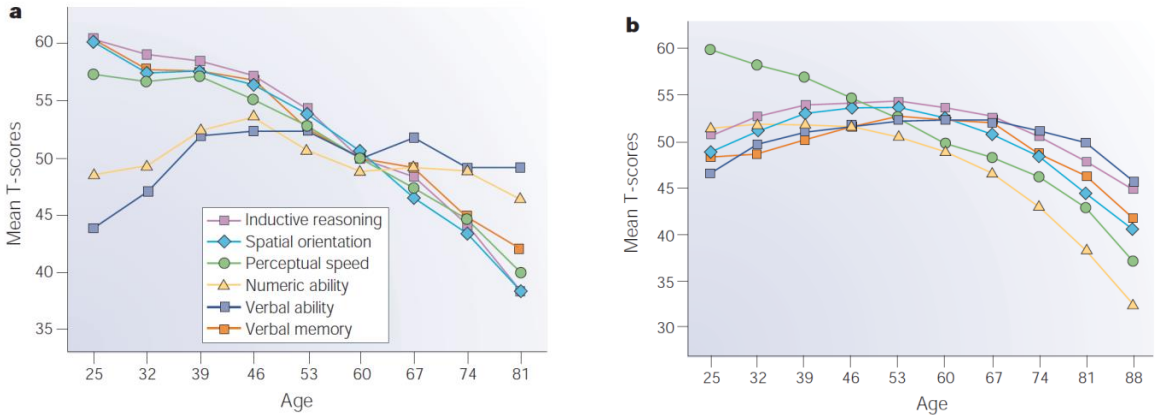


Figure 1: Cross-sectional (a) and longitudinal (b) lifespan performance across a range of cognitive domains (adapted from Hedden & Gabrieli, 2004).

Life-long declines.

Age-related decline is evident in cognitive functions associated with the effectiveness and efficiency of processing (Salthouse, 2010b). Cognitive functions including working memory, episodic memory processing speed, visuospatial skills and executive function fall into this category, as do other functions involving the manipulation of abstract or familiar information (Salthouse, 2010b). However, cross-sectional and longitudinal data yield different results with respect to when this decline begins (see Figure 1). While cross-sectional research indicates a linear decline commencing from 20 years of age, with little or no evidence of accelerated decline of these functions in later-life, longitudinal studies suggest a curvilinear age-related slope, suggesting an acceleration in decline of these functions during later-life (Hedden & Gabrieli, 2004).

Cross-sectional Verses Longitudinal Research Findings

Inferences about age-associated cognitive changes are closely related to how cognitive function is measured, with a marked discrepancy in age trends depending on whether the data was obtained through cross-sectional or longitudinal methods. However, each method has relative strengths and weaknesses. Cross-sectional studies can be influenced by time- or historical-related changes in society, such as educational opportunity, socioeconomic status and cultural factors which might create group differences within particular age cohorts (Hedden & Gabrieli, 2004; Salthouse, 2010a). While the longitudinal approach could be impacted by practice effects and may therefore underestimate change. However, the longitudinal approach enables measurement of intra-individual change (individual differences), and is therefore considered the gold-standard.

Biological Determinants of Age-related Cognitive Decline

The following discussion will outline the various structural changes that occur in the aging brain and the key genes associated with cognitive decline. While these biological factors produce small, measurable declines in cognitive function, they do not constitute dementia.

Structural Changes in the Brain

Grey matter.

Declines in grey matter characterise the course of normal aging and are due to a steady decline in synaptic densities, beginning from approximately 20 years of age (Terry & Katzman, 2001). While *in vivo* imaging studies of the brains of non-demented, healthy young and older adults reveal that older adults tend to have lower grey matter volumes (Hedden & Gabrieli, 2004; Resnick, Pham, Kraut, & Zonderman, 2003), it seems that the rate of grey matter tissue loss is similar for older and younger adults (59 – 85 years), at approximately $2.4 \pm 0.4 cm^3$ per year (Resnick et al., 2003).

Prefrontal cortex.

Similarities between the cognitive and behavioural deficits demonstrated by older adults and patients with frontal lesions indicates that functional changes in the prefrontal cortex are another source of cognitive aging (Hedden & Gabrieli, 2004). Research has shown that the lateral prefrontal cortex undergoes the greatest amount of age-related volumetric change, estimating declines of 5.36% (cm^3) per decade in healthy older adults (Raz, Gunning-Dixon, Head, Dupuis, & Acker, 1998). As well as volumetric changes, the prefrontal cortex also

experiences age-associated changes to neurotransmitters. These changes, summarised in a review by Hedden and Gabrieli (2004) involve age related declines in dopamine concentration, transporter availability and dopamine D2 receptors.

White matter.

White matter consists mainly of glial cells and myelinated axons which serve to transmit signals between regions of the brain. When comparing the white matter in brains of healthy, young and older adults, older adults show significant tissue loss (Hedden & Gabrieli, 2004; Resnick et al., 2003). The rate of white matter tissue loss appears to be similar for older and younger adults, at approximately $3.1 \pm 0.4 cm^3$ per year. Non-pathological aging seems to be characterised by decreases in white matter density primarily in the prefrontal cortex and anterior regions of the brain (Head et al., 2004). It has been proposed that the age-related loss of white matter integrity could affect the interaction between the prefrontal cortex and other structures, particularly the hippocampus (Hedden & Gabrieli, 2004).

Hippocampus.

The hippocampus and related medial temporal lobe structures only experience subtle age-related changes in healthy older adults up until the age of 50 years, with an estimated rate of volumetric decline of 0.71% per decade (Raz et al., 2004). However, between the ages of 50 and 80 years, volumetric decline increases to up to 6.38% per decade (Raz et al., 2004). These volumetric changes closely mirror the pattern of age-related memory decline, with little age-associated explicit memory decline occurring until after the age of 60.

Genetics

Twin studies and research into families with adopted children have estimated the heritability of general cognitive function to be approximately 50% (Deary et al., 2009). It is suggested that the heritability of cognitive function increases from childhood into old age (Deary et al., 2009). However, some research suggests that the influence of genetic factors on both cognitive performance and rates of cognitive decline decreases in old age (70s and 80s) (Lee, Henry, Trollor, & Sachdev 2010). The likelihood of genetic influences of cognitive function has seen research reveal two candidate genes proposed to account for variance in cognitive function and cognitive decline in older adults: apolipoprotein E (*APOE*) and brain derived neurotrophic factor (*BDNF*).

Apolipoprotein E.

One alternative form of the apolipoprotein (*APOE*) gene is the epsilon 4 allele (*APOE* ε4) and appears to have an important role in age-related changes to cognitive function. It is well established that the ε4 allele is associated with an increased risk developing AD, with carriers of at least one ε4 allele up to three times more likely to develop the disease (Mondadori, de Quervain, Buchmann, & Mustovic, 2007). This is thought to be due to a link between the ε4 allele and the pathological hallmarks of the disease. *APOE* ε4 is associated with increased risk of the formation of amyloid plaque deposits and neurofibrillary tangles (Bennett et al., 2005; Bennett, Wilson, Schneider, & Evans, 2003a; Mondadori et al., 2007).

Allelic variant of the *APOE* gene is also implicated in variance in healthy adult cognitive function. *APOE* ε4 allele carriers have shown poorer performance compared to non-carriers

in episodic memory (Deary et al., 2004; Zehnder, Bläsi, Berres, & Monsch, 2009), executive function and general cognition (Wisdom, Callahan, & Hawkins, 2011). According to Wisdom et al. (2011), these performance differences between ε4-carriers and non ε4-carriers becomes more pronounced with age. Conversely, other research indicates that cognitive performance does not vary based on ε4-carrier status (Donix et al., 2012; Jorm et al., 2007). However, few studies have examined the relationship between the *APOE* ε4 allele and the rate of cognitive change over time. Knight et al. (2014) reported no significant differences in rate of decline between *APOE* ε4 carriers and non-ε4 carriers aged over 65 over a 10 year period.

Brain derived neurotrophic factor.

Brain derived neurotrophic factor (*BDNF*) is a protein widely distributed in the human brain, particularly in the hippocampus, cerebral cortex and the amygdala (Murer, Yan, & Raisman-Vozari, 2001). *BDNF* modulates the growth of new neurons, the survival of existing neurons, as well as regulating synaptic function and plasticity (Poo, 2001). Research conducted on a polymorphism of the *BDNF* gene, *BDNF* Val66Met, has revealed that secretion of *BDNF* in neurons is impaired when the Val66 sequence is replaced with a Met sequence (Egan et al., 2003). Consequently, the Met variant of a polymorphism of the *BDNF* gene, *BDNF* Val66Met, leads to decreased availability of *BDNF* in the brain (Egan et al., 2003). Met carriers consistently display reduced hippocampal function (Egan et al., 2003) and volume (Pezawas et al., 2004) relative to their Val/Val homozygote counterparts. Carrying the Met polymorphism has also been associated with poorer performance in memory and executive function in adults with preclinical AD (Lim, Villemagne, Ellis, et al., 2014) and amnestic mild cognitive impairment (Nagata, Shinagawa, Nukariya, Yamada, & Nakayama, 2012). Conversely, Ventriglia et al. (2002) report that homozygous Val carriers are at increased risk

of developing AD. Another study found no association between *BDNF* polymorphisms and AD diagnoses (Lee et al., 2005).

Similar inconsistencies exist within research examining the influence of *BDNF* carrier status on cognitive function in healthy older adults. While some studies implicate the Met variant of the Val66Met polymorphism in decreased performance in episodic memory (Egan et al., 2003), working memory (Richter-Schmidinger et al., 2011) and processing speed (Miyajima et al., 2008) relative to Val/Val homozygotes, other studies fail to find an association between *BDNF* Met carrier status (Persson, Lavebratt, & Wahlin, 2013; Stuart, Summers, Valenzuela, & Vickers, 2014).

Candidate gene combinations.

Given the findings that variation in *APOE* and *BDNF* show independent associations with increased risk for AD and poorer cognitive performance in older adults, it is possible that these genes could interact to confer heightened risk for those who carry both the ε4 allele and *BDNF* Met. While Ward, Summers, Saunders, Janssen, et al. (2014) reported that episodic memory performance was highest in *APOE* ε2/*BDNF* Met allele carriers compared to all other *APOE* allele and *BDNF* polymorphism gene combinations, there was no evidence of a detrimental effect of *APOE* or *BDNF* combinations on cognitive performance. Further, little is known regarding whether certain genetic combinations have a beneficial or detrimental effect on cognitive functions over time.

Brain Reserve and Cognitive Reserve

While aging and genetic factors are among the biggest risk factors associated with AD and rapid age-related cognitive decline, certain environmental and lifestyle factors are also proposed to play an integral role in cognitive function and rates of age-associated cognitive decline in later-life. The proposed theoretical mechanism through which such life experience factors have a relationship with age-related cognitive decline is referred to as *cognitive reserve*.

Support for the reserve concept stems from autopsy reports which revealed a subset of individuals with the neuropathological markers of Alzheimer's disease despite having sound cognitive function prior to death (Katzman et al., 1988). Consequently, cognitive reserve is thought to act as a buffer between level of brain pathology and its clinical manifestation (Figure 2), such that the higher the level of reserve, the more severe pathology can be before functional impairment becomes evident. The concept can be separated into two theoretical approaches based on whether reserve is viewed as a passive process, or as an active process.

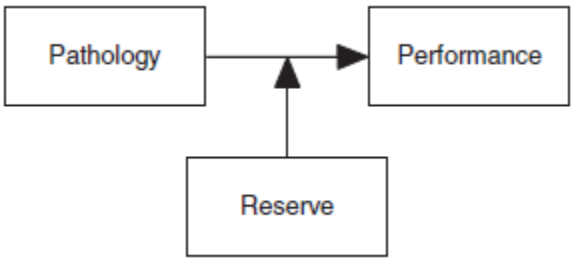


Figure 2: Reserve modifies the relationship between pathology and performance.

Brain Reserve: A Structural, Passive Model of Reserve

The theory of *brain reserve* maintains that individual differences in brain *structure* enable some people to cope better with pathology than others (Katzman, 1993). Brain reserve focuses on the potential protective features of brain anatomy, including brain size and the number of neurons and synaptic connections (Richards & Sacker, 2003; Stern, 2009). Within this approach it is believed that larger brains are able to cope with higher levels of pathology before functional impairment emerges because adequate neural substrate remains to sustain normal functioning (Stern, 2002).

Satz (1993) integrated brain reserve and threshold concepts to formulate the threshold model of reserve, which revolves around the concept of *brain reserve capacity* (BRC). The model postulates that there are individual differences in amount of BRC and that each individual has a critical threshold of BRC, such that once BRC is depleted past this critical threshold, function impairment will emerge (Figure 3). Theoretically, individual differences in BRC are associated with differences in the clinical manifestation of a particular degree of damage to the brain (Stern, 2009).

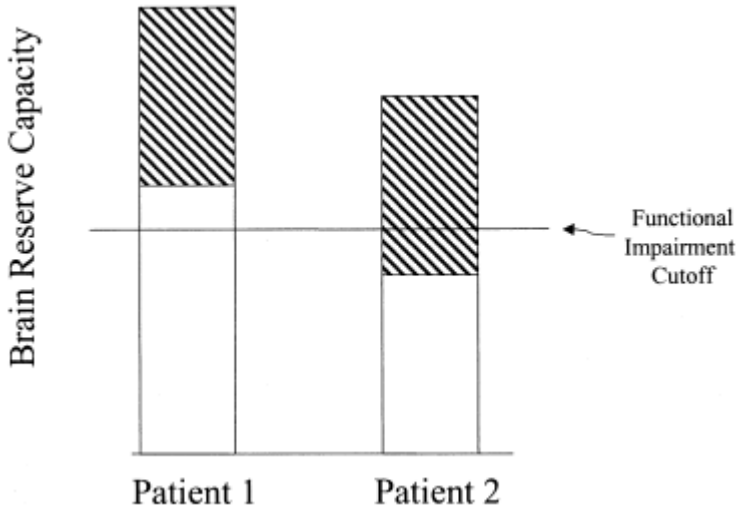


Figure 3: The threshold model of reserve (Stern, 2002).

Assumptions and limitations of brain reserve and threshold models.

Due to the inherently quantitative nature of passive models, they assume that an identical brain injury will cause the same consequences for every individual (Stern, 2009). The fundamental message is that if the level of brain damage is sufficient, it will deplete BRC below the cut-off point for functional impairment. In this sense, brain reserve models fail to account for individual differences in how the brain attempts to process cognitive and functional tasks following brain damage. Though these issues do not detract from the contribution of passive models in explaining the discrepancy between degree of brain damage and functional impairment, they suggest that extensions of threshold/passive models need to be considered in order to account for the full range of reserve.

Cognitive Reserve: A Functional, Active Model of Reserve

Active models of reserve offer an alternate theoretical approach to reserve which may account for the limitations of passive models. In active models of reserve, such as *cognitive reserve (CR)*, it is proposed that the brain actively endeavours to cope with brain damage by using pre-existing cognitive processes or by utilising compensatory processes (Stern, 2002, 2009). This model suggests that although two patients have the same amount of BRC, the patient with a greater amount of CR could tolerate more brain damage than the other patient before functional impairment becomes evident (Figure 4) (Stern, 2009).

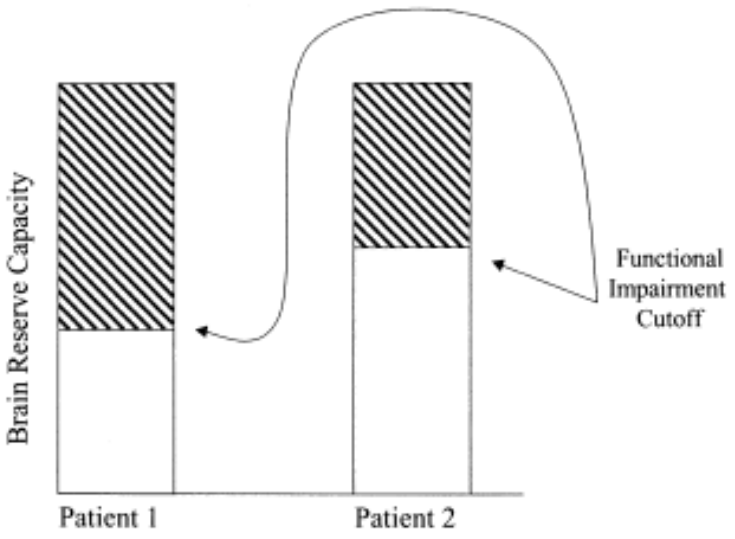


Figure 4: Cognitive reserve model (Stern, 2002).

Active models add something unique to accounts of reserve offered by passive models because they consider individual differences in the way individuals process tasks which may enable some to cope with brain damage more effectively than others (Stern, 2009). Individual differences in levels of CR can relate to genetic differences or from life experience such as occupational or educational attainment (Stern, 2003). The focus is on the processes

that enable individuals to tolerate brain damage and preserve cognitive function, rather than on the structure of the brain itself.

The measurement of cognitive reserve.

Though conceptually distinct, brain reserve and cognitive reserve are related constructs. Presumably, the cognitive processes underlying the cognitive reserve model have a physiological basis and as such it can be seen that both models imply a neural substrate that is capable of mediating cognitive performance and protecting against functional impairment (Richards & Deary, 2005; Stern, 2009). The key difference between the two models resides in the level of analysis. Brain reserve models propose individual differences in terms of the quantity of available neural substrate (Stern, 2009), such as the number of available neurons or synaptic connections. Cognitive reserve models on the other hand, imply individual differences in the ability to recruit and co-ordinate specific brain regions (Jones et al., 2011; Stern, 2009). This research thesis is focusing on cognitive reserve because it is conceptualised as a dynamic concept that can increase throughout the lifespan, as opposed to brain reserve, which is conceptualised as something that is based on fixed features of brain anatomy.

The measurement of cognitive reserve.

Although there is some underlying neural basis for CR, levels of CR are typically inferred from indirect measures, or proxy measures, of lifetime experiences (Jones et al., 2011; Stern, 2009) thought to provide reserve and thereby moderate the incidence of AD (Scarmeas & Stern, 2003).The most frequently used measures include educational attainment, occupational

attainment and leisure time activities (Jones et al., 2011). Greater levels of each reduce the incidence of AD (Valenzuela & Sachdev 2006).

However, research has shown that education, occupational attainment, and leisure activities differentially contribute to CR (Foubert-Samier et al., 2012). This suggests that research which utilises a single proxy measure to measure CR may be insufficient. Acknowledging the multivariate nature of CR, Ward, Summers, Saunders, and Vickers (2014) developed two latent measures of CR using factor analysis. Prior CR combines proxy measures traditionally associated with CR, including education and other prior life experiences, whereas current CR is a composite measure of different proxy measures designed to assess dynamic change in CR arising from exposure to new life experiences (Ward, Summers, Saunders, & Vickers, 2014). Further research is needed to validate this operational measure of CR against an intervention designed to enhance individual levels of CR.

Building cognitive reserve to reduce age-related cognitive decline.

Though the concept of CR is often used to explain individual differences in the clinical manifestation of disease, Stern (2003) acknowledges that it is equally applicable to explaining individual differences in normal cognitive function. Prospective longitudinal research supports the role of social activity (Ertel, Glymour, & Berkman, 2008; Lövdén, Ghisletta, & Lindenberger, 2005; Zunzunegui, Alvarado, Del Ser, & Otero, 2003), physical activity (Sofi et al., 2011; Yaffe, Barnes, Nevitt, Lui, & Covinsky, 2001), education (Alley, Suthers, & Crimmins, 2007; Anstey & Christensen, 2000; Bosma, van Boxtel, Ponds, & Houx, 2003) and participation in mentally stimulating activities (Ghisletta, Jean-François, & Martin, 2006; Schooler & Mulatu, 2001) in superior cognitive performance and a reduced

rate of cognitive decline over time. This superior cognitive performance occurs due the underlying increase in CR presumably associated with such activities.

Jones et al. (2011) suggests that reserve theory applies across the lifespan, from cognitive development in childhood, through to adulthood and old age. In this sense CR can be conceptualised as dynamic in nature – it is not determined only through early life experiences but can develop across the lifespan. Therefore, it should be possible to improve cognitive reserve in later-life in order to enhance cognitive function as people age, reduce rates of age-related cognitive decline, and delay the onset of AD. Consequently, there has been a strong research focus on designing lifestyle based interventions to enhance cognitive function in later-life.

Lifestyle Interventions in Later-life

The consequences of age-related cognitive decline for an individual can be debilitating and are associated with a reduced quality of life, lack of functional independence and increased mortality (James, Wilson, Barnes, & Bennett, 2011). Thus, it is of importance to identify potential interventions that can be activated in later-life for those most at risk of rapid age-related cognitive decline. The 'engaged lifestyle' or 'use it or lose it' hypothesis proposes that engagement in social, physical and intellectual activities in older adulthood prevents or reduces the decline of cognitive functions through providing complex mental stimulation across a variety of environments (Salthouse, Berish, & Miles, 2002; Stine-Morrow, Parisi, Morrow, & Park, 2008). Such theories suggest that people who engage in more cognitively stimulating activities in older age, will perform better on measures of cognitive function, experience less cognitive decline with age, and potentially reduce the risk of developing

Alzheimer's disease (Stine-Morrow et al., 2008). The findings associated with interventions designed to enhanced cognitive function in later-life will now be summarised. Specifically, they fall into two categories: physical training interventions and cognitive training programs.

Physical Training Interventions

The benefits of regular physical activity on cardiovascular health and physical function are well documented (Chang et al., 2010). There is also a growing body of research indicating some short-term and long-term benefits of physical activity for cognitive function in later-life.

An early study (Kramer et al., 1999) randomly assigned 124 previously sedentary adults (aged 60-75 years) to either an aerobic training group (walking) or an anaerobic group (stretching and toning). It was found that subjects who had received aerobic training for the 6-month period, displayed better performance on measures of executive function, compared to the anaerobic training group (Kramer et al., 1999). However, improvements were only found in the aspects of each task that were frontally mediated (Kramer et al., 1999).

Masley, Roetzheim, and Gualtieri (2009) compared the cognitive performance of a control group who completed minimal aerobic activity (0-2 days per week), a moderate aerobic group (3-4 days per week) and a high aerobic group (5-7 days per week) at baseline and again at the conclusion of the 10 week intervention program. After controlling for demographic factors including education, as well as psychomotor speed, only tests reflecting cognitive flexibility (a component of executive function) showed an improvement, which was proportional to the frequency of exercise undertaken by each individual (Masley et al., 2009).

In a meta-analysis of 18 intervention studies, Colcombe and Kramer (2003) reported that fitness training in previously sedentary older adults increased performance across a range of cognitive functions, regardless of the type/frequency of training and participant characteristics. The second major finding was that there appeared to be process-specific benefits. Tasks involving executive processes had the largest effect sizes, but controlled processes, visual-spatial processes and speed also displayed reliable benefits from fitness training.

It seems that physical interventions result in improved information processing speed and executive function. Though these results are promising, due the nature of intervention studies it is unknown whether these improvements will be maintained over time or dissolve following cessation of aerobic training. Further, it cannot be determined whether such physical interventions result in reduced rates of age-related cognitive decline in the absence of longer follow up periods. Churchill et al. (2002) posit that the benefit of short-term physical training could be limited to the cognitive functions that have shown the most age-related cognitive decline.

Cognitive Training Programs

The findings from a number of studies indicate that CR can be enhanced or modified through cognitive training programs. Cognitive training programs, sometimes called cognitive stimulation programs, are non-pharmacological interventions which broadly aim to decrease the rate of age-related cognitive decline, and perhaps even delay the clinical onset of AD

(Tardif & Simard, 2011). Generally these programs require the rehearsal of a particular task aimed at improving a particular cognitive function, such as memory (Tardif & Simard, 2011).

The short-term success of cognitive training.

Cognitive training programs have shown success in improving memory (Ball et al., 2002; Envig et al., 2010; Kirchhoff, Anderson, Barch, & Jacoby, 2012; Willis et al., 2006), generalised memory and attention (Smith et al., 2009) reasoning (Ball et al., 2002; Willis et al., 2006) and speed of processing (Ball et al., 2002; Willis & Schaie, 2006). Although there is some evidence to suggest cognitive training is generalizable to other cognitive domains (Smith et al., 2009), overall the literature reflects specific but not general benefits of training. For example, Owen et al. (2010) found no evidence of improved general cognitive function following training. The task-specific benefts of cognitive training also did not generalize to other tasks involving similar cognitive functions.

A recent review of 14 cognitive training programs revealed improvements on trained tasks measuring memory, attention, executive functions and speed of processing after targeted cognitive training (Tardif & Simard, 2011). The author notes that the generalizability of such intervention programs to everyday life activities is seldom addressed in the majority of studies yet is a key component of assessing the efficacy of such programs (Tardif & Simard, 2011). For example, whether an individual's day-to-day memory function can be improved with cognitive training.

Potential long-term benefits of cognitive training.

The ACTIVE study group is the first to retest both the cognitive and functional effects of cognitive training over the long-term (e.g. Ball et al., 2002). The ACTIVE Study Group assigned older adults (aged 65-94 years) to one of four 10 session training groups (memory, reasoning, speed of processing, or a no-contact control group). Each intervention improved the targeted cognitive skill and this improvement still persisted at the two year follow up (Ball et al., 2002). At the 10 year follow up, reasoning and speed, but not memory training had maintained the improved targeted cognitive abilities (Rebok et al., 2014). After 10 years the intervention was also associated with a lower rate of decline in self-reported instrumental activities of daily living compared to the no contact control group (Rebok et al., 2014). However, as few studies have directly examined whether these short-term gains in cognitive function can be maintained over long periods of time, further follow up studies are required before conclusions can be made.

Cognitive training in the age of the internet.

In the context of a society where older adults are the fastest growing cohort of internet users (Hart, Chaparro, & Halcomb, 2008), computerised, web-based cognitive training modalities are increasingly available. A systematic review comparing neuropsychological software training programs and classic cognitive training tasks (i.e. pencil-and-paper training on standardised tasks) was recently conducted and revealed that the results from computerised training methods were comparable and in some cases superior to those of classical cognitive training paradigms (Kueider, Parisi, Rebok, & Gross, 2012). Computerised training methods are advantageous in the sense that they are less costly, less labour intensive and can be self-

paced (Kueider et al., 2012). However, computerised training is an isolated, individual based activity and consequently, long-term adherence to such programs may be limited (Kueider et al., 2012). This is concerning, especially given the importance of social integration and social support to healthy cognitive function in older age (e.g. Seeman, Lusignolo, Albert, & Berkman, 2001; Stoykova, Matharan, Dartigues, & Amieva, 2011).

Aims of the Present Thesis and Outline of Chapters

Despite the benefits associated with cognitive training program interventions, and the strong link between education and cognitive performance in later-life (Anstey & Christensen, 2000), the potential benefit of an education based intervention in later-life has not been directly examined. The overall aim of the present thesis was to assess the potential benefit of a tertiary based education intervention on cognitive reserve and cognitive function in a group of healthy older adults over a four year period.

Chapter two provides a comprehensive review of research examining the association between education and cognitive function in older adults. With more advanced statistical methods and longer data ranges available since a previous review was conducted in this area, it was of importance to reassess whether education offers a protective effect against normal age-related cognitive decline. Chapter two as presented has been published: Lenehan, M. E., Summers, M. J., Saunders, N. L., Summers, J. J., & Vickers, J. C. (2014). Relationship between education and age-related cognitive decline: A review of recent research. *Psychogeriatrics, 15,* 154-162. doi:10.1111/psyg.12083

Chapter three provides a description of the general methodology of the Tasmanian Healthy Brain Project. Within this chapter, a study was conducted to examine whether the computerised neuropsychological tests used in the project measured the specific cognitive functions they were purported to measure, using established neuropsychological tests as a reference point. The analysis was conducted using baseline data and a series of confirmatory factor analyses. This study is included as an appendix and as presented has been published: Lenehan, M. E., Summers, M. J., Saunders, N. L., Summers, J. J., & Vickers, J. C. (2015). Does the Cambridge Automated Neuropsychological Test Battery (CANTAB) distinguish between cognitive domains in healthy older adults? *Assessment (In press).*

The main aim of chapter four was to establish whether the education intervention enhanced CR in the intervention group, relative to a group of healthy control participants. Given that many factors make differential contributions to CR (i.e. occupation, prior education, IQ, involvement in stimulating activities), this chapter utilised a previously derived multicomponent measure of dynamic CR to assess CR change over time. The analysis utilised four years of data from the THBP. Chapter four as presented is under review for publication, having been resubmitted following revisions recommended by initial review: Lenehan, M. E., Summers, M. J., Saunders, N. L., Summers, J. J., Ward, D.D., Ritchie, K., & Vickers, J. C. (2015). Sending your Grandparents to university increases cognitive reserve: the Tasmanian Healthy Brain Project (*Under review*, *Neuropsychology).*

Chapter four established that CR did indeed increase in the intervention group over time. Consequently, chapter five examined the effect of the intervention on neuropsychological function over time. Four domains of cognitive function were assessed: episodic memory, working memory, executive function and language processing. Domain scores for the four

cognitive domains were derived from a series of principle components analyses. Again, four year data was utilised in this analysis. Chapter five has been prepared as a manuscript and is ready for submission for publication: Lenehan, M.E., Summers, M.J., Saunders, N.L., Summers, J.J., Ritchie, K., & Vickers, J.C. (2015). Does enhancing cognitive reserve in older adults through further education lead to improved cognitive function: The Tasmanian Healthy Brain Project.

Chapters six through eight considered the potential influence of two candidate genes for variance in cognitive function: apolipoprotein E (*APOE*) and the val66met polymorphism of brain derived neurotrophic factor. These chapters aimed to investigate the effect of these genes on cognitive function over time and response to the intervention. All three chapters are based on the four year data from the THBP. The main aim of chapter six was to examine the influence of the *APOE* ε4 allele on longitudinal cognitive function, as well as to investigate whether *APOE* ε4-carriers displayed a different response to the education intervention compared to non-ε4 carriers. Chapter six has been prepared as a manuscript and is ready for submission for publication: Lenehan, M.E., Summers, M.J., Saunders, N.L., & Vickers, J.C. (2015). Does *APOE* allelic variation modify responsiveness to a tertiary education intervention designed to enhance cognitive reserve: The Tasmanian Healthy Brain Project

Similarly, chapter seven considered whether *BDNF* val66met polymorphisms were related to cognitive function and response to the intervention over the four year period. Chapter seven has been prepared as a manuscript and is ready for submission for publication: Lenehan, M.E., Summers, M.J., Saunders, N.L., & Vickers, J.C. (2015). Does *BDNF* Val66Met polymorphism modify responsiveness to a tertiary education intervention designed to enhance cognitive reserve: The Tasmanian Healthy Brain Project.

Chapter eight examined the potential influence of genotype combinations on the full sample, removing intervention and control group membership from the analysis. This allowed for sufficient group sizes to examine whether certain gene-gene combinations of *APOE* and *BDNF* confer increased risk or offer protection against age-related cognitive decline over time. Chapter eight has been prepared as a manuscript and is ready for submission for publication: Lenehan, M.E., Summers, M.J., Saunders, N.L., & Vickers, J.C. (2015). *APOE* and *BDNF* polymorphisms do not interact to modify cognitive performance over a 4 year period: The Tasmanian Healthy Brain Project.

Chapter 2

The Relationship Between Education and Age-Related Cognitive Decline: A Review of Recent Research

This chapter published as:

Lenehan, M. E., Summers, M. J., Saunders, N. L., Summers, J. J., & Vickers, J. C. (2014). Relationship between education and age-related cognitive decline: A review of recent research. *Psychogeriatrics, 15,* 154-162. doi:10.1111/psyg.12083

Abstract

Background: The association between level of educational attainment and cognitive performance is well-studied. People with higher education perform better across a broad range of cognitive tasks. However, there is uncertainty as to whether education moderates the trajectory of age-related cognitive decline.

Objective: This review paper addresses the potential link between education and age-related cognitive decline by evaluating relevant research published since the year 2000.

Methods: Studies reporting data on education and its association with the rate of cognitive decline across various cognitive domains were reviewed. A total of 10 studies were identified with a mean follow-up period of 7.6 years, each containing a population-based, non-demented sample.

Results: Results showed that, in the majority of studies, education did not moderate age-associated cognitive decline. The few studies that did find an association between education and decline in specific cognitive functions should be interpreted with caution due to methodological issues.

Conclusion: The literature reveals little consistent evidence that normal age-related cognitive decline is moderated by education attainment. This supports a passive theory of cognitive reserve: People with a higher level of education will continue to perform at a higher level of cognitive functioning than their lower educated peers, which may delay the onset of impairment in the future.

Introduction

Cognitive reserve theory posits that individuals possessing a greater ability to recruit and co-ordinate specific brain regions are able to cope with a higher level of brain pathology before clinical impairment is reached (Jones et al., 2011; Stern, 2009). Quantifying an individual's level of cognitive reserve typically involves inferring cognitive reserve from indirect, or proxy, measures, such as lifetime experience, educational attainment or occupation (Jones et al., 2011; Stern, 2009). It has been argued that education increases cognitive reserve through fostering the development of new cognitive strategies (Manly, Byrd, Touradji, & Sanchez, 2004).

Multiple studies indicate that educational attainment modifies the association between a direct measure of brain pathology and neuropsychological test performance (Bennett, Wilson, Schneider, & Evans, 2003b; Dufouil, Alpérovitch, & Tzourio, 2003; Rentz, Locascio, Becker, & Moran, 2010). Such findings have led some researchers to consider education to be the key protective factor against dementia (Jones et al., 2011). In a review, Valenzuela and Sachdev (2006) demonstrated that individuals with a high level of education had a 47% decrease in risk for dementia compared to those with lower level educational attainment. A recent study has confirmed that education up to year 12 has a dose-related effect on reducing risk of dementia with advancing age, irrespective of the disease burden (Brayne et al., 2010).

On the basis of such findings, subsequent studies have also examined whether educational attainment moderates the trajectory of normal age-related cognitive decline. The term 'age-related cognitive decline' is used to describe the declines shown in cognition that occur due to the normal aging process. Age-related cognitive decline is a normal (non-pathological) and expected part of the aging process. In research published between 1985 and 1999 a number

of studies report that education reduces the rate of age-related cognitive decline (Albert et al., 1995; Bennett et al., 2003b; Evans et al., 1993; Jacqmin-Gadda, Fabrigoule, Commenges, & Dartigues, 1997; Lyketsos, Chen, & Anthony, 1999; Shichita, Hatano, Ohashi, Shibata, & Matuzaki, 1986). Other studies noted that the effect of education on ageing related decline was restricted to specific cognitive domains (Arbuckle, Maag, Pushkar, & Chaikelson, 1998; Christensen, Korten, Jorm, & Henderson, 1997; Schaie, 1989). Such findings support the concept of *active cognitive reserve*, which proposes that it is individual differences in brain efficiency, flexibility or capacity which underpin task performance (Stern, 2009). Thus, increasing levels of education confer on individuals the capacity to process tasks more efficiently. As a consequence, possessing higher active cognitive reserve allows greater capacity to cope more effectively with the subtle brain changes associated with age-related cognitive decline (Stern, 2009).

Several studies published between 1985 and 1999, however, found no effect of education on rate of cognitive decline (Carmelli, Swan, LaRue, & Eslinger, 1997; Hultsch, Hertzog, Dixon, & Small, 1998). The absence of an effect of education on the rate of cognitive decline supports a *passive* model of cognitive reserve, whereby individuals with higher educational attainment will consistently perform at a higher level of cognitive function as they age because of this greater level of baseline cognitive reserve, but decline at a similar rate to their lower educated peers.

There are a number of possible explanations for these discrepant findings. In this regard, an important consideration is the potential role of cohort differences. As the age-ranges vary between studies, the age-related cognitive decline identified in some studies may be an artefact of historically related cohort differences and therefore may over-estimate age-related

decline (Hedden & Gabrieli, 2004). It has been shown that adjusting for an individual's cognitive function at baseline can contribute to a false or inaccurate association between education and change in cognitive performance (Glymour, Weuve, Berkman, Kawachi, & Robins, 2005). Whether the rate of change is calculated based on two or multiple time points is also an important consideration. Earlier research predominantly based estimates of cognitive change on only two measurement points. Such an approach is limited in its ability to estimate rate of change over time, as it is difficult to distinguish changes due to pathological or normal aging processes from changes due to learning and practice effects or random variation (Morris, Evans, Hebert, & Bienias, 1999).

The manner in which data is analysed can affect the reported outcomes. While earlier studies with two measurement points tended to adopt regression analysis (Christensen et al., 1997) or repeated measures analysis of variance (Colsher & Wallace, 1991), recent studies with three or more time points utilise more sophisticated analytical techniques, including latent growth curve modelling (Alley et al., 2007) (Christensen et al., 2001; Tucker-Drob, Johnson, & Jones, 2009; Zahodne et al., 2011), and linear mixed modelling (Der, Allerhand, Starr, Hofer, & Deary, 2010; Van Dijk, Van Gerven, Van Boxtel, Van der Elst, & Jolles, 2008). These more sophisticated techniques are better able to cope with both missing data and unevenly spaced assessment time points, which are common occurrences in longitudinal aging research.

A comprehensive review of research examining the association between education and age-related cognitive decline has not been completed since the review provided by Anstey and Christensen (2000) published 14 years ago. Twelve out of the 14 studies reviewed by Anstey and Christensen (2000) reported that education had a beneficial impact on cognitive decline.

However, since this review was published, a potential threshold effect of education has emerged with Lyketsos et al. (1999) finding that in individuals with more than 8 years of education there was no association between education and rate of cognitive decline. With the increase in the average level of education in industrialised nations, it is possible than the population wide level of education has increased since the review by Anstey and Christensen (2000). Consequently, the potential protective effects of education may no longer be evident in populations as education levels go beyond the 8 year threshold reported by Lyketsos et al. (1999). More than a decade since the previous review was published, it is of interest to examine whether more advanced statistical techniques and longer ranges of longitudinal data yield a more consistent finding regarding the potential protective benefit of education on normal age-related cognitive decline.

Method

As an existing review of the literature was published based on studies conducted up until the year 2000, studies included in this review were published in English language journals after this time. These studies included empirical data on education and its association with rate of cognitive decline in older adults (≥ 50 years of age). Studies were identified through searches in the Web of Science and Psych Info databases. The search terms "education" and "cognitive decline" and "age-related" were contained in the title, abstract or content of the article.

Inclusion criteria were: (a) one of the independent variables was education; (b) one of the outcome variables was rate of cognitive decline; (c) the study was longitudinal; (d) the study assessed participants on a minimum of two occasions; (e) the sample was initially healthy,

free from a significant health problem that could impinge on cognitive function *or* the study statistically controlled the impact of health status; (f) participants were aged 50 years and older; and, (e) cognitive function was assessed across multiple cognitive domains.

Studies were excluded if: (a) the main outcome was dementia; (b) the sample included participants with dementia or cognitive impairments (including cases of Mild Cognitive Impairment) at baseline; (c) the participant sample included chronic illness but did not statistically control for the effect of such conditions on cognitive function; or, (d) cognitive function was assessed using solely a general mental status measure. Studies which included measures of cognitive function at two time points but did not analyse rate of decline or change over time were not included in this review.

The results of each study were assessed qualitatively. The findings are discussed with reference to sample characteristics, the treatment of education as a continuous or categorical variable, the analysis method and the sensitivity of testing instruments. A meta-analysis of results was not attempted due to the range of different cognitive tests, education measures and statistical techniques adopted within the various studies.

Results and Discussion

The initial search yielded 168 articles from Web of Science and 190 articles from the Psych Info databases. The majority of studies were excluded as they did not meet all inclusion criteria. Ten studies were retained for this review that met all inclusion criteria.

Level of Education

The effect of education on cognitive performance.

Consistent with previous literature (Kramer, Bherer, Colcombe, Dong, & Greenough, 2004), all reviewed studies demonstrated that education was related to better performance across most (Christensen et al., 2001; Proust-Lima et al., 2008), if not all (Alley et al., 2007; Der et al., 2010; Seeman et al., 2005; Tucker-Drob et al., 2009; Van Dijk et al., 2008; Zahodne et al., 2011) cognitive domains (see Table 1).

Table 1: Studies examining the association between education and age-related cognitive decline.

Author & Date	n	Sample and Sampling Method	Age range Years at baseline	Education Categorical or continuous	Education Mean Years (*SD*) (When available)	Study design and analysis	Cognitive Functions	Findings
Cullum et al. (2000)	135	Subsample of the Cambridge City Over-75 Cohort. Population-based sample drawn from general practice lists	75-85+	Categorical <15 years (64%) >15 years (36%)		2 assessments over 4 years Logistic Regression	The Cambridge Cognitive Examination (CAMCOG) subscales: memory, attention/calculation (combined), perception, orientation, praxis, abstract-thought and language.	Less education associated with decline in memory subscale only. Declines occurred in all other functions but were not associated with education.
Christensen et al. (2001)	887	Canberra Longitudinal Study. Probability sample of persons aged over 70 years drawn from electoral roll, community dwelling	70-93	Continuous & <10 (*N*=68) 10-12 (*N*=127) >12 (*N*=99)		3 assessments over 8 years *1. Latent growth curve modelling* 2. ANOVA and Regression analyses on survivors for whom complete data available (*N*=294)	Crystallised intelligence (vocabulary, similarities & NART); memory (word recognition, recall of three items, address recall); speed (SLMT); general cognitive function (MMSE)	1. Education significantly related to level of CIQ, memory and speed; education level not associated with differences in rates of decline on any cognitive measure. 2. Education associated with better performance in CIQ, speed and MMSE, but not memory. Decline evident across 8 year period for speed, memory, and MMSE but not CIQ; no differences in rate of decline as a function of level of education for any function.
Bosma et al. (2003)	708	Maastricht Aging Study longitudinal data (MAAS). Convenience sample drawn from a registration network of general practices	50-80	Continuous & 3 categories ranging from primary education to university education		2 assessments over 3 years Ordinary least squares regression	Processing speed (modified Stroop-Colour-Word Test); verbal memory (Verbal Learning Test); general cognitive function (MMSE)	Low educational level associated with faster decline in speed, memory and general cognitive function when compared to a high educational level. The associations lose statistical significance when controlling for mental workload and intellectual abilities.

Seeman et al. (2005)	895	MacArthur Successful Aging Study data. Population-based sample from which a subsample drawn on the basis of age and physical and mental health.	70-79	0-8 years (reference group) (29.1%) 9-11 years (25.5%) 12 years (24%) 13+ years (21.5%)	Overall *M*=10.64 (*3.43*)	3 assessments over 7 years Mixed models	Memory (sum of delayed incidental recall and delayed spatial recognition); abstraction (4 items of Similarities); language (modified BNT); spatial ability (figures); global cognition (sum of scores on 5 tests listed above)	Higher education associated with better performance on all 5 cognitive measures. No significant differences in rates of decline as a function of education level across any function. For those with 13+ and 9-11 years education the *APOE* e4 allele was associated with faster decline in global cognition over time, similar trend observed for those with 12 years education though $p>.05$.
Alley et al. (2007)	6651	Asset and Health Dynamics of the Oldest Old (AHEAD) data. Nationally representative sample of older American's living in the community.	70+	Continuous	*M* = 11.1 (3.5)	4 assessments over 7 years Growth curve modelling	Verbal memory (delayed and immediate recall); working memory (Serial 7's); general mental status (Telephone Interview for Cognitive Status)	Higher education related to better performance on all three cognitive tests. Higher education was associated with slower decline in general mental status, faster decline in verbal memory and was unrelated to rate of decline in working memory.
Van Dijk et al. (2008)	872	MacArthur Successful Aging Study data, subsample drawn on the basis of age and cases with no missing data. Convenience sample randomly drawn from general practice registers.	49-81	Categorical Low (primary & lower vocational, ≤10 years) or High (secondary education or university)	Low: *M* = 8.3 (1.6) High: *M* =11.3 (2.9) Total sample: *M*=9.9 (2.8)	3 assessments over 6 years Linear mixed modelling	Verbal learning (The Verbal Learning Test); long-term memory (delayed recall modified RAVLT); attention switching (modification of Trail Making); semantic fluency (verbal fluency test); phonemic fluency (verbal fluency test)' interference control (Stroop Colour-Word Test); mental speed (Letter Digit Substitution Test); general cognitive status (MMSE).	Higher education related to better performance on all cognitive tests. Rate of decline did not differ depending on educational level on any of the cognitive tests.
Proust-Lima et al. (2008)	1800	Personnes Agées QUID (PARQUID) data, subsample without dementia. Convenience sample randomly selected from electrol roll	65+	Categorical Low (no primary school diploma) ~≤6 years of education (*N*=453) High (primary school diploma)~≥6 years education (*N*=1347)		8 assessments over 15 years Non-linear latent process models	Global cognitive performance (MMSE); verbal fluency (Isaacs Set Test); verbal memory (recognition form of the Benton Visual Retention Test); psychomotor speed (Digit Symbol Substitution Test); Latent cognitive factor (the common factor of the four psychometric tests)	Linear mixed models showed that subjects with higher education performed better on visual memory and psychomotor speed tasks but there were no significant differences between education groups on MMSE or verbal fluency score. Subjects with higher education declined at a faster rate for measures of global cognitive function and psychomotor speed. Non-linear models revealed that higher education was associated with faster decline in verbal fluency and psychomotor speed. There were no significant differences in rate of decline between performance

								on the MMSE or visual memory task.
Tucker-Drob et al. (2009)	690	Advanced Cognitive Training for Independent and Vital Elderly (ACTIVE), subsample no-contact control group. Convenience sample drawn from various registers and settings.	65-94 years	Continuous	Range: 6-20 years M=13.4 (2.7)	5 assessments over 5 years Latent growth curve modelling techniques	Reasoning (Letter Series, Word Series and Letter Sets); processing speed (three tasks from the field-of-view measure); vocabulary (a test from the Kit of Factor Referenced Cognitive Tests). Composite test scores representing reasoning and speed were also computed.	Education was related to cognitive performance but not associated with rates of cognitive decline over time, both before and after controlling for baseline education
Der et al. (2010)	398	Healthy Old People in Edinburgh (HOPE) study, subsample based on completion of cognitive tests. Convenience sample identified through age registers of general practices.	70+	Continuous	*M* =10.9 (2.6) at baseline	3 assessments over 9 years Linear mixed effects modelling	Fluid intelligence/non-verbal inductive reasoning (Raven's Standard Progressive Matrices) and verbal declarative memory (Logical Memory).	Participants with higher education had a higher mean score on both cognitive outcomes at baseline. There were no interaction effects between age and education suggesting there are no differences between rates of cognitive decline between those with lower or higher education levels.
Zahodne et al. (2011)	1014	Victoria Longitudinal Study, two subsamples based on follow up period. Convenience sample consisted of community-dwelling volunteers.	54-95	Continuous & categorical ≤13 years or ≥14 years education	Range 6–20 years. Sample 1 *M*=13.4 years Sample 2 *M*=14.7 years Entire sample *M*=14.1 (3.1) years	Up to 5 assessments over 12 years Unconditional and conditional growth models and	Verbal processing speed (lexical decision and sentence verification); working memory (sentence construction and two span tests); verbal fluency (three tests from the Kit of Factor Referenced Cognitive Tests: controlled associates, opposites and figures of speech); verbal episodic memory (immediate recall from two word list learning and two story memory tasks).	After controlling for age at baseline and gender, higher education was related to better performance in all cognitive domains, especially verbal fluency. The effect of education was the smallest for the processing speed domain. However, higher education was not associated with reduced rate of decline in any cognitive domain. Considering education as a dichotomous variable did not alter this pattern of results. Excluding the covariate of baseline age and running separate models in subgroups of younger (<70 years) and older (>70 years) still revealed no association between education and the trajectory of cognitive decline.

The effect of education on rate of cognitive decline

Table 1 presents the results of studies that report data on the role of education on rate of cognitive decline that met the inclusion criteria of this review. There are four patterns of findings: (1) studies that report an effect of education (e.g. a higher level of educational attainment is associated with a slower rate of cognitive decline) (Bosma et al., 2003); (2) studies that found an effect of education but only in particular subgroups (Seeman et al., 2005); (3) studies that report an education effect restricted to some cognitive functions but not others (Alley et al., 2007; Cullum, Huppert, McGee, & Dening, 2000; Proust-Lima et al., 2008); and (4) studies that report no association between education and rate of cognitive decline (Christensen et al., 2001; Der et al., 2010; Tucker-Drob et al., 2009; Van Dijk et al., 2008; Zahodne et al., 2011).

Global cognitive function

When considering measures of global cognitive functioning, the selected studies demonstrate inconsistent findings. Bosma et al. (2003) and Alley et al. (2007) found that higher levels of education were associated with slower decline in global cognitive function. In contrast, Proust-Lima et al. (2008) report that individuals with higher education declined at a faster rate. However, when repeating the same analysis using non-linear modelling techniques, there was no significant difference in the rate of decline in global cognitive performance between lower and more highly educated individuals (Proust-Lima et al., 2008), suggesting that analytical method could be an influential factor when exploring cognitive change trajectories through ageing. Similarly, Christensen et al. (2001), Seeman et al. (2005) and

Van Dijk et al. (2008) found no significant differences in rates of decline in global cognition in older people as a function of education. However, when looking specifically at those individuals with the *APOE* e4 allele (Seeman et al., 2005), a non-significant trend ($p. < 0.1$) for a faster decline in global cognitive performance was found in individuals with greater educational attainment (>9 years).

Screening measures of global cognitive function are common to most studies included in this review, particularly the use of the Mini Mental State Examination (MMSE; Folstein, Folstein, & McHugh, 1975) However, it is important to consider the appropriateness of using a measure of general cognitive function in assessing age-related cognitive decline. The MMSE was designed to provide a brief measure of cognitive status in adults and as a screen for cognitive impairment (Monroe & Carter, 2012), but is not effective at distinguishing either subtle subclinical changes in cognition from normal performance (Galasko et al., 1990) or in the adequate assessment of specific cognitive domains (Lezak, Howieson, Bigler, & Tranel, 2012). The MMSE also lacks the sensitivity to robustly assess non-memory domains (Alladi, Arnold, Mitchell, Nestor, & Hodges, 2006), such as processing speed, a function which demonstrates significant decline in late adulthood (Hedden & Gabrieli, 2004). Research examining age-related cognitive decline highlights differential trajectories of deterioration for specific cognitive functions (Hedden & Gabrieli, 2004). Consequently, there is a need for research to focus on specific cognitive functions using established psychometric techniques in order to explore the potential protective effects of education. The MMSE may also be insensitive to change among high-functioning or well-educated adults (Jacqmin-Gadda et al., 1997). Due to these factors, the findings from studies utilising global cognitive screening measures should be interpreted with caution. Interpreting the results from neuropsychological

tests with established sensitivity and specificity in assessing specific cognitive domains is more informative in this context.

Specific cognitive functions

The majority of studies reviewed do not report significant differences in the rate of cognitive decline between lower and more highly educated individuals on measures of verbal memory (Christensen et al., 2001; Der et al., 2010; Seeman et al., 2005; Van Dijk et al., 2008; Zahodne et al., 2011), visual memory (Proust-Lima et al., 2008), processing speed (Christensen et al., 2001; Tucker-Drob et al., 2009; Van Dijk et al., 2008; Zahodne et al., 2011), spatial ability (Cullum et al., 2000; Seeman et al., 2005), abstract thought/reasoning (Cullum et al., 2000; Der et al., 2010; Tucker-Drob et al., 2009), attention/calculation (Cullum et al., 2000) or interference control (Van Dijk et al., 2008). Such findings suggest that education does not reduce the rate of age-related cognitive decline across a range of specific cognitive domains. These findings support a *passive* model of *reserve* (Stern, 2002).The theory of *passive cognitive reserve* or *brain reserve* maintains that it is individual differences in brain anatomy, including brain size and number of neurons and synaptic connections, that determines task performance (Stern, 2009). The passive cognitive reserve model posits that age-related cognitive decline will occur at a similar rate regardless of the amount of education an individual attains throughout their life (see Figure 5a). However, of the ten reviewed studies, four report results not consistent with passive cognitive reserve explanations.

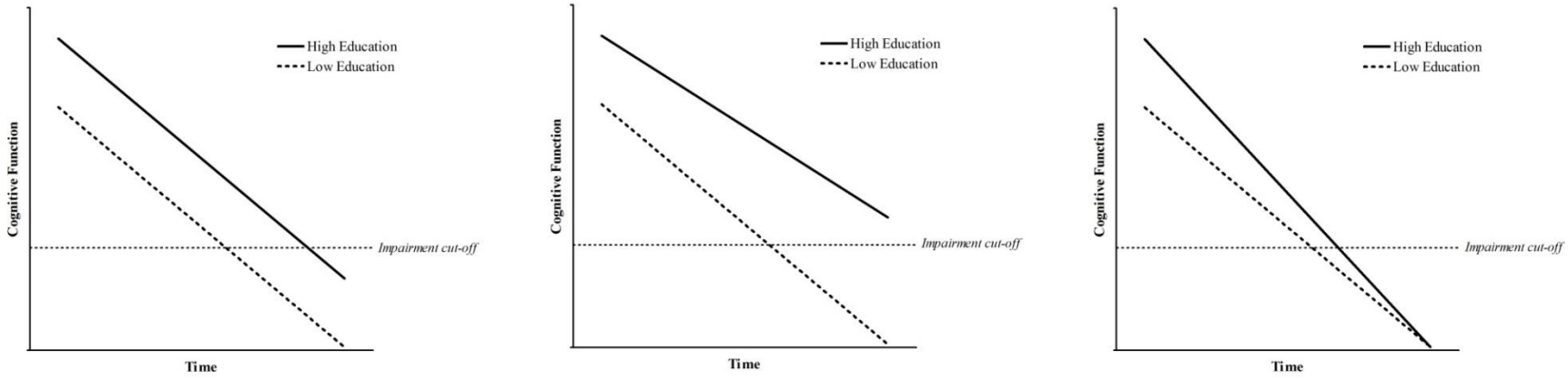
Bosma et al. (2003) report that a lower level of education was associated with more rapid decline in measures of processing speed and verbal memory. Similarly, Cullum et al. (2000)

report that age-related decline in memory was associated with lower levels of educational attainment. These findings support the notion of a *neural cognitive reserve,* which posits that higher levels of educational attainment lead individuals to process cognitive tasks more effectively and as such the structural changes associated with an aging brain are associated with reduced rates of cognitive decline relative to individuals with a lower level of education (see Figure 5b). Interestingly, the associations found by Bosma et al. (2003) disappeared when the influence of mental workload of current job and intellectual abilities were controlled, leading the researchers to suggest that accelerated cognitive decline could be due to a lack of mental stimulation at work among people with lower levels of educational attainment.

The findings of Bosma et al. (2003) and Cullum et al. (2000) however, should be interpreted with caution for two key reasons. Firstly, age-related cognitive decline is a progressive long-term process with some cognitive functions showing minimal decline over a 5-10 year period (see Hedden & Gabrieli, 2004). The studies conducted by Bosma et al. (2003) and Cullum et al. (2000) involved testing participants on only two occasions over three and four years, respectively. This may not be a sufficient time period over which to accurately model rates of cognitive change. Secondly, as there were only two time points of data more sophisticated statistical approaches, such as growth curve modelling (e.g. Zahodne et al., 2011), linear mixed modelling (e.g. Van Dijk et al., 2008) and non-linear latent process modelling (e.g. Proust-Lima et al., 2008) were not utilised in these studies. These approaches are statistically powerful and highly flexible in terms of the ability to manage both missing data and unevenly spaced assessment time points, which are common occurrences in longitudinal research, in comparison to traditional analytic methods (Curran, Obeidat, & Losardo, 2010).

Conversely, two studies reported that a higher level of education was associated with an increasingly rapid decline in cognitive function, evident on measures of verbal memory (Alley et al., 2007) as well as processing speed and verbal fluency (Proust-Lima et al., 2008). These findings are in keeping with the *neural compensation* model of *cognitive reserve* (Stern, 2009), which posits that it is individual differences in the ability to enlist alternate brain structures or networks when faced with brain pathology that underlie task performance. In line with this explanation, individuals with a higher level of educational attainment may deal with normal age-associated decline in some cognitive functions by utilising other intact cognitive domains, effectively reducing the rate of cognitive decline until these secondary functions too begin to decline (see Figure 5c).

Figure 5: Trajectories of age-related cognitive decline according to three theories of cognitive reserve



The lack of consistent evidence to support the assumption that education moderates age-related cognitive decline is inconsistent with findings from earlier research (see review by Anstey & Christensen, 2000). This could be due in part to either an insufficient number of participants with low levels of education (low levels of cognitive reserve), or an insufficient sample of individuals with very high levels of education (high cognitive reserve). For example, a sample that is mostly high-functioning at baseline could limit the statistical power of an analysis because initial test scores are higher at baseline. Although age-related cognitive decline will be apparent, greater declines may be evident in a more educationally representative sample. Previous research focusing specifically on a sample with lower levels of education found that while having 8 years of education was associated with a reduced rate of decline compared to those with <8 years of schooling, having ≥9 years of education provided no additional protective effect (Lyketsos et al., 1999).

Unfortunately, most of the research reviewed does not clearly specify the number of participants at the lower and higher extremes of education. Of the studies reviewed, a few include a substantial proportion of participants with lower levels of education (Christensen et al., 2001; Seeman et al., 2005; Van Dijk et al., 2008), providing evidence contradictory to the claim of Lyketsos et al. (1999). However, as the majority of research in this field examines participant samples with an average of >10 years educational attainment, there remains scope for further research into how education effects cognitive decline in lower education groups (see Table 1).

Cohort effects may also help to explain the lack of association between education and reduced age-associated cognitive decline found in the most recent studies. Historically, educational attainment has steadily increased in developed counties such as the United States

of America since the early 1900s (Hester, Kinsella, & Ong, 2004). If cognitive reserve is enhanced with education and life experience, it is possible that with increasing levels of educational attainment as the norm, the majority of the population may attain maximum cognitive reserve capacity. If this occurs, the capacity to detect the effect of increased education on cognitive reserve may dissipate in the future as variance in educational attainment disappears. If a sample individuals with lower levels of education (e.g. <8 years) was studied, a protective effect of education may emerge, which would be consistent with the findings from research in the 1980s and 1990s (Anstey & Christensen, 2000).

The departure away from an education effect found in earlier research may also be due to the more advanced analytical techniques now available to researchers. Earlier research tended to rely on more rudimentary analysis methods such as regression analysis or analysis of variance techniques and report a protective effect of education on rates of cognitive decline over time (e.g. a reviewed in Anstey & Christensen, 2000). The impact of using more sophisticated modelling techniques in order to monitor individual change over time is evident in research. In the analyses of cognitive change between time one and time two data (see Christensen et al., 1997), which utilised regression, an education effect was found. However, when three time points were analysed using latent growth modelling (Christensen et al., 2001) this protective effect of education was no longer evident.

Of the papers that use modelling techniques, only three mention the specific time metric used (e.g. time since study entry or age). The time metric used can change the interpretation of results, particularly if age at entry was the time metric. This is because the intercept of the age needs to be set at a certain point, consequently, if the centring point are different between studies then the curves represent different reference points. Of these three studies included in

the review that specify which time metric was used, age-centring occurred, but was centred at different ages. In one study, the intercept represented the baseline mean score at age 63 (Van Dijk, Van Gerven, Van Boxtel, Van der Elst, & Jolles, 2008), in the second study the intercept represented the baseline mean score at age 79 (Der, Allerhand, Starr, Hofer, & Deary, 2010), and the third represented the baseline mean score at age 70 (Zahodne et al., 2011). Consequently, the curves created in these models have different references points. Nonetheless, these three studies all did not find a significant effect of education on age-related cognitive decline.

A limitation of this review is that the Pubmed database was not utilised in the initial search for relevant articles. In addition, it is acknowledged that other search terms may also have yielded appropriate articles, such as "cognitive trajectory" or "cognitive change". Consequently, the initial search parameters may have been too strict and potentially failed to reveal other possibly relevant articles. A further limitation of this review was the strictness of the inclusion criteria, which excluded individuals with major health problems that could impact cognitive function. Consequently, the sample may not accurately reflect the general health of older adults and elderly living in the community.

Implications and Conclusions

This review demonstrates that there is little consistent evidence to support the assumption that education moderates age-related cognitive decline across any cognitive domain(s) in healthy older adults. The limited evidence supporting this association must be interpreted with caution due to current methodological constraints, including short study duration and the statistical analysis technique used, and potential cohort effects. Substantial evidence exists indicating that individuals with higher levels of educational attainment will continue to

perform at a higher level of cognitive functioning when compared to their lower educated peers. Such results suggest support for a passive model of cognitive reserve. However, in the absence of studies directly examining the relationship between education and cognitive decline in healthy older adults with low levels of education, it remains possible that the relationship between education and rate of decline varies according to the level of education of an individual. In this sense, education is beneficial because more highly educated individuals will continue to perform at a higher level of cognitive functioning as they age and may withstand neurodegenerative pathology for a longer period of time before functional or clinical impairment is reached. However, there appears to be no decrease in the rate of age-related cognitive decline over time attributable to increased levels of education.

Another consideration neglected in existing research is measures of effect size. Effect sizes are useful in distinguishing trivial results from those with practical or clinical significance (Tabachnick & Fidell, 2013). This is particularly important in the context of what are often large sample sizes in population based aging research. In studies involving large sample sizes, statistical significance may have little practical meaning (Lantz, 2013). The research reviewed does not explicitly report effect sizes, and, in many cases, does not report the data necessary to calculate effect sizes. An analysis of the magnitude of particular significant education effects may reveal only small effect sizes with little practical utility. This may help to explain the inconsistency evident in the findings of different studies.

The effect of education among lower educated groups (<8 years) remains under-researched. Further investigations are required to determine if lower levels of education are associated with greater rates of age-associated cognitive decline. If this is the case, low-level educational subpopulations may benefit most from education-based intervention approaches. The effect

of education in later stages of adulthood remains to be explored. Future research should examine the effect beyond that attained during a person's initial schooling. This approach could also assist in the development of interventions designed to target those groups most at risk of rapid age-related cognitive decline.

Chapter 3

The Tasmanian Healthy Brain Project: General Methodology

Sections of this chapter published as:

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Structure and Design

The Tasmanian Healthy Brain Project (THBP) is a world first prospective study examining whether university level of education in later-life delays age-related cognitive decline. The study adopts a mixed-group longitudinal design. There are two groups:

1. *Control group*: Healthy adults who did not engage in any university level education during the study.
2. *Intervention group*: Healthy adults who undertook a minimum of 12 months part-time or full-time study, with a minimum load of one unit per semester for two consecutive semesters. Units of study could be from an undergraduate or postgraduate program. A typical undergraduate Bachelor degree at the university where the participants were enrolled required completion of 24 units of study.

Participants

Participants were invited to become involved in the Tasmanian Healthy Brain Project through advertisements in print and radio media, public talks and lectures, the local newspaper, through referrals from existing participants and through information sent out through university bulk emailing lists.

All participants in both the control and intervention groups were community dwelling and aged between 50 and 79 at the time of recruitment into the study. Potential participants were initially screened for conditions independently associated with cognitive function and excluded from entry into the study if: there was known dementia; multiple sclerosis; prior head injury requiring hospitalisation; epilepsy; cerebrovascular complications including

stroke, aneurysm or transient ischaemic attacks; poorly controlled diabetes; poorly controlled hypertension or hypotension; other neurological disorders including cerebral palsy or spina bifida; chronic obstructive pulmonary disease; heart disease; partial or total blindness; deafness; and current psychiatric diagnosis.

As of December 31st 2014, when data was extracted from the database for the present analysis 565 adults had commenced participation in the THBP (see Table 2) (Summers et al., 2013). Of these, 41 cases were excluded - one deceased, and 40 due to having withdrawn from the project prior to any follow-up testing (7% drop-out rate). As many of the neuropsychologial tests are norm-referenced from individuals who speak English as a primary language, a further 19 cases were excluded due to English being a second language. The initial sample thus contained 498 healthy older adults.

Table 2: Recruitment flow for the THBP (as of December 31st 2014)

Group	Completed Baseline Testing	Withdrawn	Deceased	NESB	Medical Exclusions	Remaining
Control	179	14	0	7	2	156
Intervention	388	26	1	12	7	342
Total	567	40	1	19	9	498

Note: NSEB = Non-English Speaking Background.

Group Sizes

The smaller size of the control group relative to the experimental group reflects the initial plan of analyses conceived by the Chief Investigators of the THBP, which was to conduct analyses based on grouping all participants by study load completed: 0 units of study (control group); 2–8 units of study; 9–16 units of study; 17–24 units of study; >24 units of study. Such groupings would lead to relatively even group sizes. However, the current thesis did not examine study load as an independent variable and consequently, group sizes are not equal. However, the benefit of the statistical approach adopted in the current thesis (latent growth curve modelling) is that group sizes are not required to be equal (Curran et al., 2010).

Sample Bias

It is acknowledged that the lack of randomisation of the two groups (experimental or control) could lead to an allocation bias. It is possible that baseline variables, such as age and education, or other factors not measured within the THBP protocol, may influence the results. In addition, random allocation of participants to groups would make the effect of intervention able to be determined with less bias than longitudinal trials.

Consequently, in all data chapters preliminary analyses were conducted to investigate whether there were significant differences between the control group and the experimental group in terms of all demographic baseline variables. When and if significant differences were found, a series of correlational analysis were performed in order to investigate whether these variables correlated with the various outcome measures across all time points. If

necessary, these factors were included in the statistical models as covariates in order to control for their influence.

It is also worth noting that while randomised control trials are considered the gold standard when measuring the effect of an intervention, they are not always appropriate. For example, it would be misguided and unethical to limit an individual's social activity for the purpose of research or in the case of the THBP, to limit access to further education.

Materials

Participants in the THBP completed a comprehensive testing battery involving assessment of cognitive reserve, neuropsychological/cognitive function, psychosocial function and genetic analysis (see Table 3).

Table 3: Tasmanian Healthy Brain Project test battery

Cognitive/Neuropsychological	***Executive Function***	**Psychosocial**
Global	COWAT (Controlled Oral Word Association Test)	HADS (Hospital Anxiety and Depression Scale)
DRS-2 (Mattis Dementia Rating Scale 2nd Edition)	RVP (Rapid Visual Processing, CANTAB)	PWI (Personal Wellbeing Index)
	MTS (Match to Sample, CANTAB)	LSNS (18-item Lubben Social Network Scale)
Episodic Memory	RTI (Reaction Time, CANTAB)	
PAL (Paired Associates Learning, CANTAB)	STROOP (Stroop Colour-Word Test)	**Confounds**
RAVLT (Rey Auditory Verbal Learning Test)		Medical health status questionnaire
LM (Logical Memory Test, WMS-III)	**Cognitive Reserve**	
RCFT (Rey Complex Figure Test)	***Prior CR***	**Genetic**
	WTAR (Wechsler Test of Adult Reading)	*APOE* (apolipoprotein E)
Working Memory	LEQ (Life Experience Questionnaire)	*BDNF* (Brain derived neurotrophic factor)
SSP (Spatial Span, CANTAB)		
DSP (Digit Span, WAIS-III))	***Current CR***	
SWM (Spatial Working Memory, CANTAB)	WAIS-III_SF1 (WAIS-III Short Form)	
LNS (Letter Number Sequencing, WAIS-III)	WRAT PMV(Wide Range Achievement Test, 4th Edition, Progress Monitoring Version	
Language		
VOC (Vocabulary, WAIS-III)		
COM (Comprehension, WAIS-III)		
BNT (Boston Naming Test)		

*WAIS-III = Wechsler Adult Intelligence Scale, 3rd Edition; CANTAB = Cambridge Automated Neuropsychological Test Battery; STROOP = 24 item Victoria version

Psychosocial Tests

Anxiety and depression.

The Hospital Anxiety and Depression Scale (HADS; Snaith, 2003) is a 14 item self-report scale designed to measure states of anxiety (HADSa) and depression (HADSd). The anxiety and depression subscales provide valid and reliable assessments of the severity of emotional state as well as screening individuals for potential emotional disorder (Snaith, 2003).

Personal Wellbeing Index.

The Personal Wellbeing Index (PWI) assesses quality of life and is an 11 item self-report questionnaire (International Wellbeing Group, 2006). Individuals are required to rate their level of satisfaction in relation to different aspects of their life, such as “How satisfied are you with your life as a whole?”.

Lubben Social Network Scale.

The 18-item Lubben Social Network Scale is a self-report questionnaire used to assess an individual’s perceived level of social support (Boston College, 2011). The scale comprises three subscales which question the size, closeness and frequency of contact with reference to the respondents’ family, neighbours and friends (Boston College, 2011).

Medical Health Questionnaire.

Information regarding health, medical conditions, prescription medication use and drug and alcohol use for the preceding 12 months was obtained using a structured questionnaire (Saunders & Summers, 2010; Summers & Saunders, 2012; Summers et al., 2013). The questionnaire was used to monitor potential confounds that may impact cognitive performance across each assessment session. The Medical Health Questionnaire consists of 33 items and included demographic information as well as items about medical conditions (e.g. heart disease, stroke, TIA, diabetes, neurological disorder), vision and hearing, the type and frequency of use of alcohol and drugs; and prescription medication use, for the preceding 12 months.

Cognitive Reserve

Two approaches to assessing cognitive reserve were used in the THBP (Table 3). The first approach involved measuring cognitive reserve prior to the education intervention (prior CR) and the second approach was adopted to measure dynamic change in cognitive reserve following the intervention (current CR). Current CR and prior CR were calculated for each participant using factor analysis defined regression coefficients as developed and described by (Ward, Summers, Saunders, & Vickers, 2014).

Prior CR.

The tests included in the calculation of prior CR were as specified in (Ward, Summers, Saunders, & Vickers, 2014): the Wechsler Test of Adult Reading (WTAR; The Psychological

The Psychological Corporation, 2001) to estimate pre-morbid intellectual capacity; five sub-scores from the Life Experience Questionnaire (LEQ; Valenzuela & Sachdev, 2007) (Young Adulthood Specific and Non-specific; and the Midlife Specific, Non-specific and Continuing Education Bonus) to quantify previous lifetime experience in education, occupation and leisure activities; and the Medical Health Questionnaire (Summers et al., 2013) to obtain each individuals' total years of prior education. The equation to calculate prior CR = .370 (WTAR FSIQ) + .408 (Prior education in years) + .567 (LEQ Young Adulthood Specific) + .565 (Young Adulthood Non-specific) + .630 (LEQ Midlife Non-specific) + .875 (LEQ Midlife Continuing Education Bonus) + 1.004 (LEQ Midlife Specific) (Ward, Summers, Saunders, & Vickers, 2014).

Current CR.

The tests included in the calculation of current CR were as specified in Ward, Summers, Saunders, and Vickers (2014): Wechsler Adult Intelligence Scale, 3rd edition, Short Form 1 (WAIS-III-SF1; Donnell, Pliskin, Holdnack, Axelrod, & Randolph, 2007) to estimate current intellectual capacity and the spelling and math computation subtests of the Wide Range Achievement Test, 4th edition, Progress Monitoring Version (WRAT-4-PMV; Roid & Ledbetter, 2006) to assess current academic ability. The WRAT-4-PMV has four alternate versions of each test, which were utilised to avoid learning effects (e.g. Form 1 at baseline, Form 2 at year 1 follow up). The equation used to calculate current CR = .454 (WAIS-III-SF1) + .369 (WRAT-4-PMV Spelling LES) + .463 (WRAT-4-PMV Math Computation LES) (Ward, Summers, Saunders, & Vickers, 2014).

Neuropsychological/Cognitive Function

The neuropsychological/cognitive battery comprised a combination of traditional pencil and paper administered tests, as well as computer based tests using subtests from the Cambridge Automated Neuropsychological Test Battery (CANTAB). All tests were selected on the basis of excellent reliability and validity, as well as their suitability to be used at 12 month retest intervals (Cambridge Cognition Limited, 2012; Lezak et al., 2012; Strauss, Sherman, & Spreen, 2006). The tests selected have previously been shown to be sensitive to detecting subtle declines in function associated with age-related cognitive decline, Mild Cognitive Impairment and AD (Cambridge Cognition Limited, 2012; Lezak, Howieson, & Loring, 2004; Petersen et al., 1999; Saunders & Summers, 2010; Summers & Saunders, 2012).

Global cognitive function.

The *Mattis Dementia Rating Scale, 2nd edition* (DRS-2; Jurica, Leitten, & Mattis, 2001) is a 38 item instrument which provides an objective measure of dementia severity, as well as screening for individuals with possible dementia. The DRS-2 has excellent utility and validity in diagnosing dementia (Jurica et al., 2001). Participants selected to take part in the present study displayed a DRS-2 AEMSS score ≥9; which is above the cut-off for clinical dementia and is consistent with intact general cognition.

Memory and learning.

The Logical Memory test.

The Logical Memory test (LM; Wechsler, 1997b) assesses the capacity to hold information in both short-term and long-term memory. Participants are read two brief narratives and then required to recall themes and details from the stories, both immediately (LMI short-term memory trial) and again after an approximate 20 minute delay (LMII long-term memory trial). The average internal reliability of the LM across all age groups is high (LMI r = .88 and LMII r = .79) and in older age-groups the test-retest reliability of the LM is also high (LMI r = .8 and LMII r = .76) (Wechsler, 1997b). The LM test has been validated against its correlations with other tests of memory and factor analytic studies indicate that LM loads on a verbal memory factor (Wechsler, 1997b).

The Rey Auditory Verbal Learning Test.

Verbal episodic memory was also assessed using the *Rey Auditory Verbal Learning Test* (RAVLT; Lezak et al., 2012). The RAVLT is a verbal list learning and memory test in which 15 words are presented repeatedly across five successive trials. Following each list presentation participants verbally recall as many words as possible from the list. To assess long-term memory function, an interference trial is then administered following which participants must then recall as many words as they can from the initial list. The internal co-efficient alpha of the RAVLT is r = .90 and test-retest over one year intervals ranges from r = .60 - .70 (Strauss et al., 2006). The RAVLT correlates moderately with other measure of memory such as LM, visual reproduction and the Californian Verbal Learning Test and factor analytic studies support that the RAVLT loads primarily with verbal memory tests (Strauss et

al., 2006). In addition, the RAVLT is able to detect memory decline in early AD and MCI (Saunders & Summers, 2010; Summers & Saunders, 2012)..

Paired Associates Learning test.

The *Paired Associates Learning* (PAL) test assesses visual episodic memory and learning (Cambridge Cognition Limited, 2012). The test requires participants to recall the spatial location of a predetermined number of unique patterns within a display matrix. The stimulus array becomes increasingly challenging on successive trials. The PAL has shown a high level of test-retest reliability in elderly samples (r = .80-.89) and factor analytical studies demonstrate that the test loads heavily onto a learning and memory factor (Strauss et al., 2006).

The Rey Complex Figure Test.

The *Rey Complex Figure Test* (RCFT; Strauss et al., 2006) is designed to evaluate visuospatial constructional ability and visual memory. Participants are required to firstly copy a complex geometric figure (RCFT copy) and then reproduce it from memory following a five minute delay (RCFT delay). In the context of the THBP, the RCFT was used to specifically assess visuospatial memory. The split-half reliability and Cronbach's alpha are adequate (copy trial: r > .60; recall trial: r > .80) (Strauss et al., 2006). The test-retest reliability of the PAL in a group of older adults assessed annually over a three year period ranged from low to adequate (copy trial: r = .56 -.68; recall trial: r = .57 -.77) (Strauss et al.,

2006). Factor analytic studies indicate that the test loads heavily with Wechsler Memory Scale visual reproduction, on a visuo-spatial perceptual memory factor (Strauss et al., 2006)

Working memory.

Digit Span test.

The Digit Span (DSP) subtest of the Wechsler Adult Intelligence Scale, 3rd edition (WAIS-III; Wechsler, 1997a) was used to assess short-term capacity for auditory-verbal information. In the DSP subtest, number sequences are presented verbally to the participant. The participant is then required to repeat the numbers in the same order (DSP forward), and on a subsequent trial in the reverse order (DSP backward). The DSP has high levels of reliability with an internal reliability coefficient of $r = .90$ and test-retest reliability of $r = .89$ in an older age group (Wechsler, 1997a). Factor analytic research indicates that it loads well onto a working memory factor (Wechsler, 1997a).

Letter Number Sequencing.

The *Letter-Number Sequencing* (LNS) subtest, also from the WAIS-III (Wechsler, 1997a), assesses the participants' ability to hold and manipulate auditory-verbal information in short-term memory prior to recall (Lezak et al., 2012). Participants are verbally presented with a series of numbers and letters in a random order, they repeat these letters and numbers but in a specific order: numbers first in numerical order and then the letters in alphabetical order. The LNS has high levels of reliability with an internal reliability coefficient of $r = .82$ and test-

retest reliability of r = .80 in an older age group (Wechsler, 1997a). Factor analytic research indicates that it loads well onto a working memory factor (Wechsler, 1997a).

Spatial Span.

The Spatial Span (SSP) test assesses the capacity to hold visual information in short-term memory, and is a computerised version of the Corsi Blocks task (Cambridge Cognition Limited, 2012). The task requires participants to remember and recall a sequential series of coloured boxes in the correct order. The sequence length increases throughout successive trials. The longest requires participants to remember the order of appearance of 9 coloured squares. Has marginal test-retest reliability (r = .60-.68) and factor analytic studies have shown that it loads onto an executive processes factor (Strauss et al., 2006).

Spatial Working Memory.

The *Spatial Working Memory* (SWM) subtest assesses the ability to manipulate spatial information and strategy use in short-term memory (Cambridge Cognition Limited, 2012). In this task, participants use a process of elimination to find a blue token hidden inside each one of an array of boxes. Successive trials of the SWM add additional boxes to the stimulus array, making the task increasingly more demanding on visual working memory. Has marginal test-retest reliability (r = .60-.68) and factor analytic studies have shown that it loads onto an executive processes factor (Strauss et al., 2006).

Language.

Vocabulary.

The *Vocabulary* subtest of the WAIS-III assesses the capacity to comprehend and verbally express words (Wechsler, 1997a). Common words in the English language are read out individually to the participant. After each word is read out, the participant is asked to explain the meaning of the word. For example, "repair" and "encumber". The words are of increasing complexity, and include both concrete and abstract concepts. This Vocabulary test is resistant to age-related cognitive decline and performance does not decline until the late stages of dementia (Lezak et al., 2004). The Vocabulary subtest has demonstrated a high level of test-retest reliability in an older age group: r = .93 (Wechsler, 1997a). Factor analytic studies show that it loads well onto a language factor (Wechsler, 1997a). In addition, the Vocabulary test is resistant to age-related cognitive decline and performance does not decline until the late stages of dementia (Lezak et al., 2004).

Comprehension.

The *Comprehension* subtest of the WAIS-III assesses the capacity to use language to verbally express comprehension of social conventions and proverbs, as well as the ability to solve practical problems (Lezak et al., 2012). Participants are read aloud a series of questions or statements and are required to provide a verbal response. Items include questions such as, "Why should people pay taxes?". The subtest has an high internal consistently, r = .84 and test-retest reliability r = .85 (Wechsler, 1997a). Factor analytic studies show that it loads well onto a language factor (Wechsler, 1997a).

Boston Naming Test.

The Boston Naming Test (BNT) assesses the capacity to name common and uncommon objects presented visually (Lezak et al., 2012). Participants are given a book of 60 black and white sketches and are asked to name the object in each picture. If no response is provided, thematic and phonemic clues are given at the examiners discretion but attract a lower score. The BNT has demonstrated moderate to high levels of reliability: internal coefficient alpha *r* = .78 - .96; and test-retest reliability in healthy older adults at 1-2 week interval *r* = .90 (Strauss et al., 2006). The BNT correlates highly with other language related measures including: visual naming, semantic fluency, verbal comprehension, and verbal IQ (Spreen & Strauss, 1998).

Executive function.

Controlled Oral Word Association Test.

The *Controlled Oral Word Association Test a*ssesses the ability to spontaneously produce words starting with a specific letter within 60 seconds. Participants are required to name words that begin with the letter ‘f’, ‘a’ and ‘s’ in three successive trials. Proper nouns are excluded. The test taps into the individual’s capacity to produce fluent speech and think flexibly, while also controlling impulses. The COWAT has moderate to high reliability with the internal between letters F, A and S at *r* = .83 and test-retest correlations above *r* = .70 (Strauss et al., 2006). The COWAT correlates highly with other phonemic and semantic fluency tests, verbal IQ and working memory functions (Strauss et al., 2006) and is sensitive to early changes in cognitive function (Lezak et al., 2004).

Rapid Visual Processing.

The *Rapid Visual Processing* (RVP) test assesses visual sustained attention (Cambridge Cognition Limited, 2012), which is a component of executive function. Participants view a white box in the middle of the screen in which numbers from 2-9 appear in a pseudo-random order. Within this array, participants are required to detect three different target number sequences (e.g. 3-5-7). The RVP is sensitive to subtypes of MCI and AD (Saunders & Summers, 2010).

Match-to-Sample.

The *Match to Sample Visual Search* (MTS) is a pattern matching test, requiring rapid and accurate responses. As such, inherent to the task is a speed/accuracy trade-off (Cambridge Cognition Limited, 2012). Participants are required to find the exact match for a target pattern from an array of patterns that are similar in terms of colour and shape. The MTS is sensitive to both MCI and AD (Saunders & Summers, 2010).

Reaction Time.

The *Reaction Time* (RTI) subtest from the CANTAB battery assesses attention and measures both the speed of decision making and response time. There are five stages to the task, with increasingly complex response chains (Cambridge Cognition Limited, 2012). In each stage, the participant responds as soon as they see a yellow dot appear. The dot may appear in one of five locations across the screen.

The Stroop Colour-Word Test.

The *24 item Stroop Colour-Word Test* measures the speed of information processing and impulse control for auditory–verbal information (Lezak et al., 2012). There are three stages to the test, each of increasing difficulty. In Stroop A, participants are required to name the colours of a series of dots. In Stoop B, individuals must name the colour that the stimulus words are printed in. In the final version, Stroop C, while the task is still to name the colour of the ink, this time the words are incongruent colour names (e.g., "green" is printed in red ink) (Strauss et al., 2006). The Stroop has excellent internal reliability (internal coefficient between r = .90 and .91) and correlates well with other measures of attention including: Trail Making A and B, working memory tests and the Paced Auditory Serial Edition Test (PASAT) (Strauss et al., 2006).

Trail Making.

There are two elements of the Trail Making Test. Trail Making Test Part A (TMT-A) assesses visual search speed and information processing speed. It involves connecting 25 encircled numbers which are spread across a page. Participants are required to connect the numbers in order with a continuous line while under the pressure of time (Strauss et al., 2006). The Trail Making Test Part B (TMT-B) adds the element of mental flexibility by integrating numerical and alphabetical information. To complete the task, participants must alternate between the numbers 1-13 and the letters A-L, connecting them in order with a continuous line, as quickly as possible (Strauss et al., 2006). The tests have a high level of internal reliability (Part A r = .79 and Part B r = .89) (Strauss et al., 2006). The Part A and Part B tests correlate with each other, and also correlate well with other measures of visual

search, visual sequencing and speed, such as digit symbol coding and the Wisconsin Card Sorting test (Strauss et al., 2006).

Genotyping

DNA was extracted from saliva samples using Oragene DNA Self-Collection KITS (DNA Genotek Inc., 2012) through a procedure previously used in the THBP (Stuart et al., 2014; Ward, Summers, Saunders, Janssen, et al., 2014). Briefly, *BDNF* genotype was determined using a one-step amplified refractory mutation system polymerase chain reaction (ARMS-PCR). The method described by (Sheikha, Hayden, Kryski, Smith, & Singha, 2011) was used to determine Val66Met. *APOE* genotype was determined using a one-step amplified refractory mutation system polymerase chain reaction (ARMS-PCR). The method described by Donohoe, Pulkki, Kairisto, Salomäki, and Lehtimäki (1999) was used to determine rs429358 and rs7412PCR. PCR amplifications were undertaken in a 12 μl reaction volume that contained approximately 50 ng of genomic DNA. PCR amplicons were resolved on 2% agarose gel. Genotyping was repeated on samples to ensure accuracy.

Procedure

After obtaining consent, the test battery was administered to each participant in the following order: WTAR, DRS-2, Medical Health Questionnaire, LEQ; WAIS-III SF1, WRAT-4-PMV, PAL, RAVLT, RCFT copy, Logical Memory I, RCFT recall, SSP, Digit Span, SWM, Letter Number Sequencing, Logical Memory II, Vocabulary, Comprehension, BNT, COWAT, RVP, MTS, RTI, STROOP C, TMT B, HADS, PWI, LSNS and concluding with DNA

collection. An approximate 20 minute delay occurred between the administration of LMI and LMII. LEQ and WTAR data and a DNA sample were only collected once, at baseline.

DNA was collected using Oragene DNA collection kits and required participants to spit 2ml of saliva into a collection vessel which was then combined with preservative fluid for subsequent analysis. Participants were instructed not to eat or drink (with the exception of water) for a period of 30 minutes prior to DNA collection. The full THBP test battery took approximately four hours to complete and subjects were encouraged to take short breaks as needed to avoid fatigue (Summers et al., 2013). Participants were reassessed at one year intervals (± one month).

Chapter 4

Sending your Grandparents to university increases cognitive reserve: the Tasmanian Healthy Brain Project

This chapter has been submitted for publication with minor revisions following initial review as:

Lenehan, M. E., Summers, M. J., Saunders, N. L., Summers, J. J., Ward, D.D., Ritchie, K., & Vickers, J. C. (2015). Sending your Grandparents to university increases cognitive reserve: the Tasmanian Healthy Brain Project (*Under review, Neuropsychology).*

Abstract

Objective: Increasing an individual's level of cognitive reserve (CR) has been suggested as a non-pharmacological approach to reducing an individual's risk for Alzheimer's disease. We examined changes in CR in older adults participating over 4 years in the Tasmanian Healthy Brain Project.

Method: A sample of 459 healthy older adults aged between 50-79 years. Participants underwent a comprehensive annual assessment of current CR, neuropsychological function and psychosocial factors over a four year period. The intervention group of 359 older adults (M = 59.61, SD = 6.67 years) having completed a minimum of 12 months part-time university study were compared against a control reference group of 100 adults (M = 62.49, SD = 6.24) who did not engage in further education.

Results: Growth Mixture Modelling demonstrated that 44.3% of the control sample showed no change in CR whereas 92.5% further education participants displayed a significant linear increase in CR over the 4 years of the study. These results indicate that older adults engaging in high level mental stimulation display an increase in CR over a 4 year period.

Conclusions: Increasing mental activity in older adulthood may be a viable strategy to improve cognitive function and offset cognitive decline associated with normal aging.

Introduction

One non-pharmacological approach to reducing the risk of rapid age-related cognitive decline and Alzheimer's disease is to increase cognitive reserve (CR). CR is a theoretical construct describing the capacity of an individual to utilise pre-existing brain networks efficiently (neural reserve) as well as to enlist alternate brain networks (neural compensation) when under the duress of brain pathology (Stern, 2002; Tucker & Stern, 2011). Life experiences and innate intelligence are proposed to impart CR on individuals (Stern, 2002). Research evidence supports the role of occupational attainment (Valenzuela & Sachdev 2006), intelligence (Whalley et al., 2000), education (e.g. Anstey & Christensen, 2000) and involvement in cognitively stimulating activities (Scarmeas & Stern, 2003) in modifying an individual's risk for dementia. It is inferred that the modification of an individual's risk for dementia is a result of modifications to the level of CR that an individual displays.

CR is a theoretical construct, therefore, it is imperative to recognise that what is measured (latent variable, observed score on a task or test, or physical property) is not the same thing as the construct (Zumbo, 2007). At best, attempts to operationalise and measure CR (Harrison et al., 2015) represent proxy measures with differing levels of construct validity. Various studies have used single proxy measures to infer the impact of CR on cognitive performance and rate of age-related cognitive decline. For example, individuals with lower occupational status have shown lower performance on measures of global cognitive function in later-life (Dartigues, 1992; Frisoni, Rozzini, Bianchetti, & Trabucchi, 1993; Jorm, Rodgers, Henderson, & Korten, 1998). Similarly, a socially engaged lifestyle in later life is associated with superior cognitive performance and a reduced rate of age-related cognitive decline (Barnes, Mendes de Leon, Wilson, & Bienias, 2004; Ertel et al., 2008; Lövdén et al., 2005).

A key contributor to CR is thought to be education. Education is seen as increasing CR through fostering the development of new cognitive strategies (Manly et al., 2004). Educational attainment is not only associated with a decreased risk of dementia (Valenzuela & Sachdev 2006) but also modifies the association between a direct measure of brain pathology and performance on measures of cognitive function (Bennett et al., 2003b; Dufouil et al., 2003). Despite mixed results, higher levels of education in early adulthood have been associated with superior performance on measures of cognitive function (Anstey & Christensen, 2000; Lenehan, Summers, Saunders, Summers, & Vickers, 2015). Therefore, regardless of whether education influences the rate of normal age-related cognitive decline, enhancing an individual's level of cognitive function has the potential of preserving normal cognitive function for a longer period of time in the presence of neuropathological changes in the brain.

A recent advancement in the area of CR research has been the development of a multidimensional proxy measure of CR (Ward, Summers, Saunders, & Vickers, 2014). Previous research typically utilises a single proxy measure, such as years of education or occupational attainment, to infer an individual's level of CR. However, this approach may not be accurate given that education, occupational attainment, and leisure activities differentially contribute to CR (Foubert-Samier et al., 2012). Acknowledging the multivariate nature of CR, we developed two factor analysis defined latent proxy measures of CR (Ward, Summers, Saunders, & Vickers, 2014). Prior CR combines proxy measures traditionally associated with CR, including education, pre-existing intellectual capacity, and five sub-scores from the Life Experience Questionnaire (Valenzuela & Sachdev, 2007). However, as CR theoretically develops in response to new life experiences throughout the lifespan, we developed a second proxy measure of CR designed to assess dynamic change in CR (Ward, Summers, Saunders,

& Vickers, 2014). This measure of current CR incorporates cognitive tests suitable for repeated assessment including current intellectual capacity and academic ability (Ward, Summers, Saunders, & Vickers, 2014). While prior CR enables CR set earlier in life to be determined, current CR enables possible increases in CR following an intervention to be quantified. University study typically involves complex mental and social stimulation that is increasingly being accessed by older populations.

The Tasmanian Healthy Brain Project (THBP) is a world-first prospective study examining the potential of university level of education in later-life to reduce age-related cognitive decline (Summers et al., 2013). The THBP has recruited a sample of older adults, aged 50-79 years at commencement in the study, from the island state of Tasmania, Australia. The THBP adopts a mixed-group longitudinal design, comparing older adults who engaged in later-life tertiary study with a control reference group who do not undertake further education. The THBP undertakes annual assessment of each participant examining cognitive reserve, neuropsychological/cognitive function, psychosocial function and genetic factors. This paper examines whether engaging healthy older adults in university-level education results in a measureable change in CR when accounting for pre-existing CR levels for each individual.

Method

Participants

Data from participants in the THBP as of the 31 December 2014 was utilised for this study. The initial sample comprised 566 adults aged between 50 and 79 years enrolled in the THBP (Summers et al., 2013). Of these, 19 cases were excluded from the analysis due to English

being a second, rather than primary, language. A further 41 cases were excluded from analysis due to having withdrawn from the project prior to any follow-up testing. Of the remaining 498 participants, a further 39 were missing data necessary to calculate prior CR score. As prior CR was used as a covariate in the analysis participants with missing data on this variable were excluded. The final sample used in the analysis consisted of 459 healthy older adults.

Participants were not randomly allocated to conditions, but volunteered to participate in either the intervention or control conditions. Participants in the intervention group (N = 359) undertook a minimum of 12 months part-time or full-time university study, with a minimum study load of two units at undergraduate or post graduate levels. The remaining 100 subjects in the control reference group did not engage in any tertiary level study. Participants who presented with a medical, neurological, or psychiatric disorder that could potentially influence neuropsychological test performance were precluded from entry into the THBP. The project was approved by the Human Research Ethics Committee (Tasmania) Network and further details of the study protocol have been published elsewhere (Summers et al., 2013).

Materials

Participants in the THBP completed a comprehensive testing battery. For the full project protocol refer to Summers et al. (2013). The Dementia Rating Scale, 2nd edition (DRS-2; Jurica et al., 2001), the Hospital Anxiety and Depression Scale (HADS; Snaith, 2003) and the Medical Health Status questionnaire (Summers et al., 2013) were administered to ensure participants were free from dementia and of sound psychological and physical health. The

Personal Wellbeing Index (PWI; International Wellbeing Group, 2006) and the 18-item version of the Lubben Social Network Scale (LSNS; Boston College, 2011) are self-report questionnaires and were administered to assess quality of life and perceived social support within the sample.

Prior CR.

The tests included in the calculation of prior CR were as specified in Ward et al. (2015): the Wechsler Test of Adult Reading (WTAR) (The Psychological Corporation, 2001) to estimate baseline intellectual capacity; five sub-scores from the Life Experience Questionnaire (LEQ) (Valenzuela & Sachdev, 2007) (Young Adulthood Specific and Non-specific; and the Midlife Specific, Non-specific and Continuing Education Bonus) to quantify previous lifetime experience in education, occupation and leisure activities; and the Medical Health Questionnaire (Summers et al., 2013) to obtain each individuals total years of prior education.

Current CR.

The tests used for the calculation of current CR as specified in Ward et al. (2015) were: the Wechsler Adult Intelligence Scale, 3rd edition, Short Form 1 (WAIS-III-SF1) (Donnell et al., 2007) to estimate current intellectual capacity and the spelling and math computation subtests of the Wide Range Achievement Test, 4th edition, Progress Monitoring Version (WRAT-4-PMV) (Roid & Ledbetter, 2006) to assess current academic ability. The WRAT-4-PMV has four alternate versions of each test which were utilised to avoid learning effects (e.g. Form 1 at baseline, Form 2 at year 1 follow up).

Procedure

The elements of the test battery used in the current analysis were as follows: WTAR, DRS-2, Medical Health Status, LEQ, WAIS-III-SF1, WRAT-4-PMV, HADS, PWI, and LSNS. The LEQ and WTAR IQ estimate were only collected once, at baseline. Retesting occurred at one-year intervals (± one month). When available alternate versions of tests were used to minimise familiarity effects, for example, forms 1-4 of the WRAT were utilised. The full THBP took approximately four hours to complete and subjects were encouraged to take short breaks as needed to avoid fatigue (Summers et al., 2013).

Analysis

Calculating prior CR and current CR.

Current CR and prior CR were calculated for each participant using factor analysis defined regression coefficients as developed and described by Ward and colleagues (Ward, Summers, Saunders, & Vickers, 2014). The equation to calculate prior CR = .370 (WTAR FSIQ) + .408 (Prior education in years) + .567 (LEQ Young Adulthood Specific) + .565 (Young Adulthood Non-specific) + .630 (LEQ Midlife Non-specific) + .875 (LEQ Midlife Continuing Education Bonus) + 1.004 (LEQ Midlife Specific). The equation used to calculate current CR = .454 (WAIS-III-SF1) + .369 (WRAT-4-PMV Spelling LES) + .463 (WRAT-4-PMV Math Computation LES). As the regression based formula for prior CR and current CR are based on z-score transformed raw scores; current CR scores for years 1, 2 and 3 (retesting) were z-transformed against the mean and SD of the entire sample at baseline (year 0). Therefore, positive CR scores represent an increase in CR relative to baseline CR scores.

Modelling approach.

Growth Mixture Modelling (GMM) was conducted using Mplus 7.0 (Muthén & Muthén, 1998-2012) maximum likelihood with robust standard errors estimation. GMM identifies unobserved, homogenous subgroups of individuals from larger heterogeneous populations, on the basis of similar response patterns (Muthén & Muthén, 1998-2012). This is important given research has shown that various subpopulations exist within a broader population and are differentially impacted by an intervention (Jackson & Sher, 2005). This is particularly relevant in the field of CR research, given that a potential increase in CR could depend on each individual's untapped CR capacity. Taking this into account, the conventional latent curve growth approach to analysis could oversimplify and potentially underestimate change (Jung & Wickrama, 2008). As such, GMM was conducted on the control and intervention groups separately to examine whether each group is characterised by classes of individuals with distinct patterns of change in current CR.

The procedure outlined by Jung and Wickrama (2008) for conducting GMM was followed. As the number of unobserved groups is unknown to the investigator, the suggested procedure is to identify the best fitting single-class latent growth curve model (e.g. linear or quadratic) and then progressively test models with more classes until the model fit is no longer improved by the addition of extra classes (Jung & Wickrama, 2008). In all models time was paramatised with scores that represented years since study entry (0, 1, 2, 3 for the linear term and 0, 1, 4, 9 for the quadratic term). Initially, Mplus default parameters were used. The intercepts of the outcome variable at the four time points were fixed at zero. The intercepts, residual variances and covariances of the growth factors were estimated and not held equal across classes. The model allowed for the effect of the covariates on the growth parameters for each class to be estimated. Incremental model changes such as fixing growth factor

variance to zero were also investigated to find the best fitting model. In each group, initial status of the model represented mean current CR at baseline, the linear term of the quadratic trajectory represented the linear rate of change at the intercept, and the quadratic term represented acceleration/deceleration. As the models included a covariate (conditional models) the intercepts describe the growth factors (i.e. initial starting point, linear term and quadratic term) after taking into account the effect of prior CR, so these are reported throughout.

Model evaluation.

In the initial latent growth curve analysis (single-class), model fit was assessed by considering a range of fit indices: the likelihood-ratio chi-square, the root mean squared error of approximation (RMSEA), standardised root mean square residual (SRMR) and comparative fit index (CFI). As a general rule a smaller chi-square indicates a better fit. A RMSEA value <.05 and a SRMR <.05 is seen to indicate a good fitting model (Geiser, 2013). The CFI should be larger than .95. For GMM, the optimal number of classes was determined by considering both the Bayesian information criteria (BIC) and the sample adjusted BIC. As a general rule the model with the smallest information criterion is preferred (Geiser, 2013). The interpretability of classes was also considered with reference to theory and prior research (Schaie, 1989).

Results

Descriptive Data

Data from a sample 459 participants was included in this study. Participants at commencement in the study were 50 – 79 years of age, of average intelligence (WTAR est. FSIQ), free from dementia, and not clinically depressed or anxious (Table 4). The control group was significantly older ($t_{(496)} = 4.32$, $p. < .001$), more educated ($t_{(496)} = -2.68$, $p. < .01$), and had lower current CR at baseline ($t_{(494)} = -3.05$, $p. < .01$), compared to the intervention group. However, as there were no significant correlations between age or education and current CR at any time point in either the control group or the intervention group, the decision was made not to include either as a covariate in further analysis. However, as there were no significant correlations between age or education and current CR at any time point in either the control group or the intervention group, the decision was made not to include age as a covariate in further analysis. There were no significant differences between the control and intervention groups across baseline measures of prior CR, global cognition, estimated premorbid IQ, level of anxiety or level of depression. The mean scores of current CR of the control group were lower at baseline compared to the experimental group, but both groups appeared to increase current CR score overtime.

Table 4: Sample demographic and CR as a function of group.

	Control *N* at T0 = 100 M *(SD)*	Intervention *N* at T0 = 359 M (*SD*)	*Independent samples t-test* *p.*	Obtained effect size (*d*)	Power
Female *N* (%)	64 (61%)	273 (69.5%)	(χ^2) = .10		
Baseline Age	62.62 *(6.34)*	59.48 *(6.69)*	< .001	.482	.828
Prior Education	13.50 (*2.66*)	14.30 (*2.67*)	<.01		
DRS-2 AEMSS	11.81 *(2.27)*	11.96 *(2.07)*	.52	.069	.004
WTAR (est. FSIQ)	112.23 *(5.10)*	112.65 *(5.47)*	.47	.079	.005
HADS - Anxiety	5.51 *(2.91)*	5.24 *(3.15)*	.35	.090	.006
HADS - Depression	2.86 *(2.28)*	2.38 *(2.26)*	.05	.212	.076
Prior CR	-.36 *(2.27)*	.13 *(2.28)*	.06	.215	.081
Current CR					
T0 -Baseline	-.26 *(1.01)*	.07 *(.98)*	.002	.332	.354
T1	-.05 *(1.12)*	.32 *(1.05)*	.04	.341	.384
T2	.11 *(.97)*	.34 *(1.00)*	.11	.234	.108
T3	.22 *(1.11)*	.68 *(.98)*	.01	.439	.716

DRS-2 AEMSS = Mattis Dementia Rating Scale age and education corrected Mayo scaled score; WTAR (est Full Scale Intelligence Quotient) = Wechsler Test of Adult Reading Scale estimated full scale IQ; HADS = Hospital Anxiety and Depression Scale; CR = cognitive reserve.

In the control group the best fitting single class model was a linear model with prior CR included as a time-invariant covariate (χ^2(7, N= 100) = 23.00, p. = < .01, RMSEA = .15, CI (.09, .22), SRMR = .04, CFI = .95). In the intervention group the best fitting model was a quadratic model with prior CR included as a covariate (χ^2(7, N= 359) = 26.45, p. = < .001, RMSEA = .09, CI (.05, .13), SRMR .04, CFI = .98). Zero variance in the linear and quadratic

growth factors was specified to avoid an inadmissible model due to negative residual variances. These models were used to progressively test models with more classes in each of the control and intervention groups.

GMM Control Group

The lowest ABIC corresponded to a two class model. The entropy was calculated at .60 which indicated that the model had a reasonable classification of individuals into classes. Class 1 (maintainers) comprised 44.3% of the control group. In class 1, the linear slope was not significant, indicating that linear change in current CR did not significantly differ from zero (Figure 6 and Table 5). The remainder of the control group were in class 2 (improvers; 55.7%). This class had a significant linear slope suggesting progressive increase in CR over the four year period (Figure 6 and Table 5). The effect of prior CR was consistent in both classes (Table 5). Higher prior CR was associated with a higher current CR score at baseline. Prior CR did not have a significant association with the rate of linear change in current CR over time. Considering the low entropy value in the control group (.60), consideration was given to identifying common features of those individuals whose classification probabilities were borderline (close to .50). However, there were only 6 such individuals and consequently, there were insufficient numbers to see common features.

The classes were examined to determine if other demographic variables could account for class membership. However, there were no differences between maintainers and improvers in sex, age, level of depression, level of anxiety, personal wellbeing, or social connectedness.

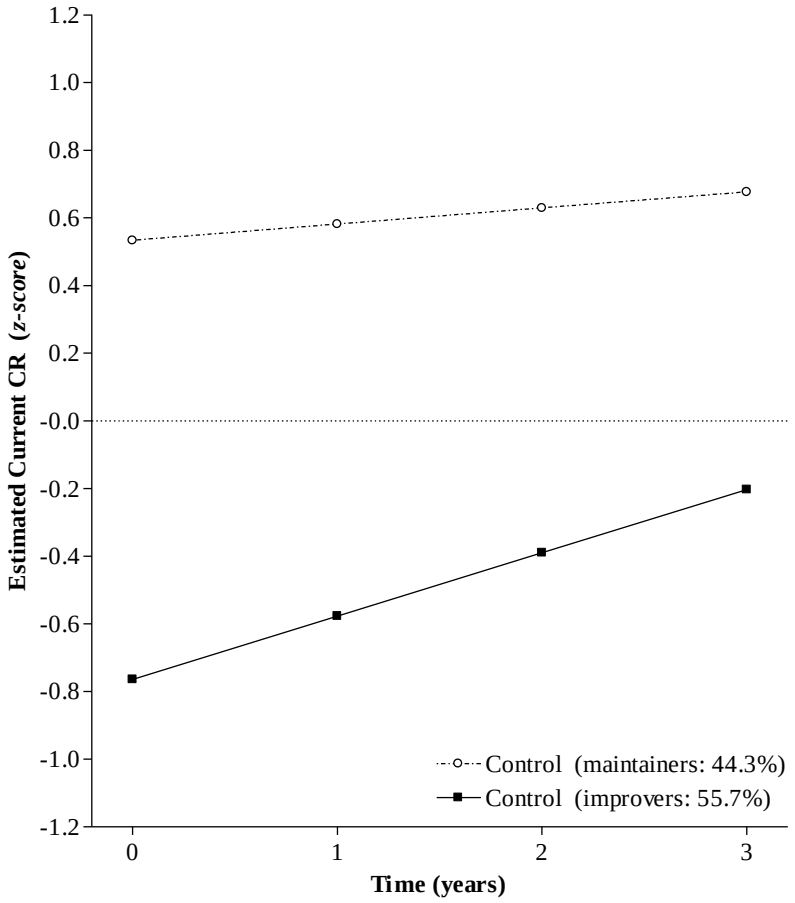

Figure 6: Control group 2 class model estimated means adjusted for the effect of prior CR *(dotted horizontal line indicates the 50th percentile of current CR of the entire cohort at baseline).*

Table 5: Estimates (S.E.) of class specific intercept parameters and the effect of prior CR on class specific growth terms for the control group.

	Class 1: Maintainers (*n* =43)	**Class 2: Improvers (*n* = 57)**
	Model estimates (*SE*)	**Model estimates (*SE*)**
Initial status	.598 (.242)*	-.674 (.114)**
Linear growth rate	.040 (.044)	.185 (.052)**
Covariate		
Prior CR		
Initial status	.180 (.078)*	.253 (.058)**
Variance	.254 (.144)	.254 (.144)
Linear term	-.022 (.019)	-.004 (.018)
Variance	.021 (.013)	.021 (.013)

Note: * *p.* < .05, ** *p.* < .01.

GMM Intervention Group

The lowest ABIC corresponded to a two class model in the intervention group also and the entropy value of .78 indicated good separation of individuals into classes. Class 1 (maintainers) constituted a minority of the intervention group (7.5%). In this class the significant, negative linear growth term indicates that current CR score decreased over the four year period and the significant quadratic term suggests that CR change accelerated over time (Figure 7 and Table 6). The majority of the intervention group were in class 2 (improvers; 92.5%). The significant linear growth term indicates that the current CR for this class increased over the 4 year period (Figure 7 and Table 6). The negative quadratic term indicated the rate of increase decelerated over time, though this parameter was not significant

(Table 6). Within Class 1 (maintainers) higher prior CR was associated with lower current CR at baseline. However, within Class 2 (improvers) higher prior CR was associated with higher current CR at baseline. In both classes, prior CR had no association with the rate of linear or quadratic change in current CR over time (Table 6). The classes were examined to see whether other demographic variables could describe class membership. However, there were no differences between maintainers and improvers in sex, age, level of depression, level of anxiety, personal wellbeing or social connectedness.

It is important to emphasise that the intervention group Maintainers was comprised of only 15 individuals. Of these 15 people, 8 had complete information for all four time points, four had data for three time points, one person had completed data at two time points and the final two people had completed just baseline assessment. Consequently, only 8 individuals contributed data to the estimation of the quadratic term and consequently the results must be interpreted with caution.

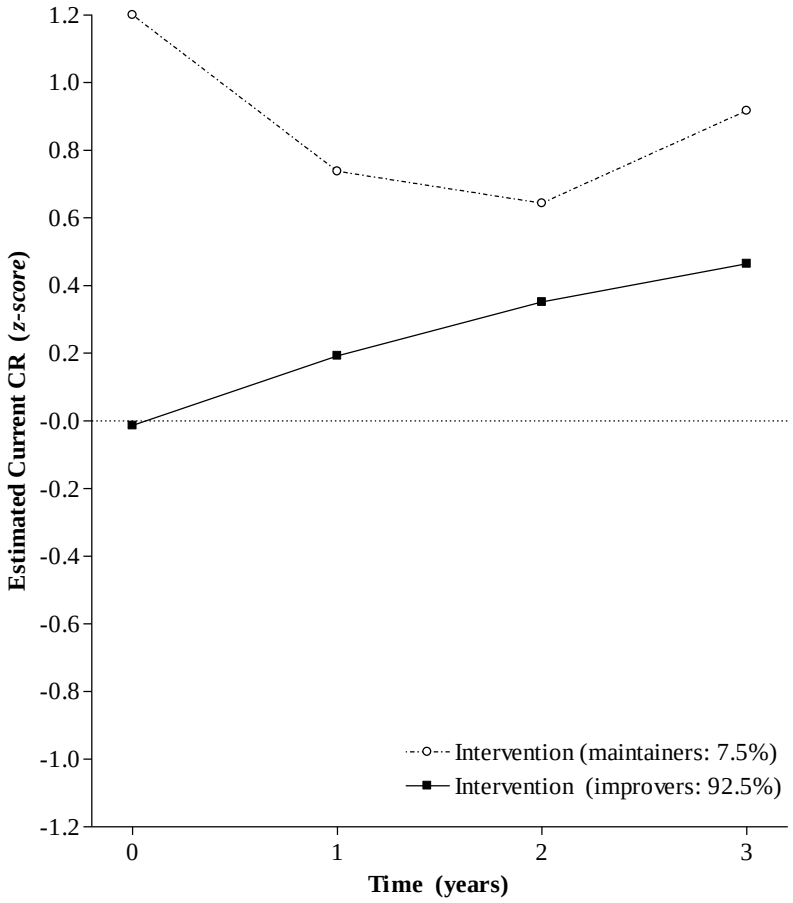

Figure 7: Intervention group 2 class model estimated means adjusted for the effect of prior CR *(dotted horizontal line indicates the 50th percentile of current CR of the entire cohort at baseline).*

Table 6: Estimates (S.E.) of class specific intercept parameters and the effect of prior CR on class specific growth terms for the intervention group.

	Class 1: Maintainers	**Class 2: Improvers**
	Model estimates (*SE*) (*n* = 15)	**Model estimates (*SE*) (*n* = 344)**
Initial status	1.227 (.229)**	-.038 (.053)
Linear growth rate	-.664 (.203)	.226 (.051)**
Quadratic growth rate	.189 (.072)**	-.022 (.018)
Covariate		
Prior CR		
Initial status	-.208 (.062)**	.182 (.022)**
Variance	.600 (.052)**	.600 (.052)**
Linear term	.133 (.082)**	.024 (.021)
Variance	.000 (.000)	.000 (.000)
Quadratic term	-.037 (.028)	-.005 (.008)
Variance	.000 (.000)	.000 (.000)

Note: * $p. < .05$, ** $p. < .01$.

Discussion

The hypothesis that individuals who receive an education intervention will display an increase in CR compared to a control group was supported by the results of the study. In both the control group and the intervention group there appear to be two distinct subgroups of individuals. In the intervention group approximately 92.5% of the sample displayed a significant increase in CR over time, while the remaining 7.5% generally maintained CR across the four year period. Among the intervention group, the maintainers displayed higher levels of CR at baseline relative to the improvers. In contrast, among the control group participants, 44.3% displayed no change in CR over time, with the remaining 55.7%

displaying a significant increase in CR over the four years. The increase in CR seen in this subgroup of control participants was evident in those individuals who displayed below average CR at baseline. Despite increasing over time, the level of CR of the control improvers remained below the 50th percentile of the baseline CR of the entire cohort.

These results indicate that the overwhelming majority of healthy older adults who engage in some degree of university level education for at least 12 months display a measureable increase in CR over a 4 year period. The small number of participants who displayed no change in CR over time while attending university already had higher than average CR at baseline (~ 1.2 SD above the cohort at baseline). This tentatively suggests that individuals with already high levels of current CR may lack the capacity for further increases in current CR. This finding should be interpreted with caution, however, due to the small sample size for this group (n = 15).

Age was explored as a potential covariate in these models. However, as age was not correlated with the outcome variables (prior CR and current CR), nor did it significantly improve the fit of the model, or dramatically alter the structure of the model, the decision was made not to include age as a covariate.

The findings of the present research are consistent with other investigations reporting benefits from cognitive training programs (Ball et al., 2002) and physical activity (Kramer et al., 1999) on cognitive function, presumably through the positive effect these activities have on building CR. The proportion of the control group who showed improvement in current CR despite not receiving the intervention is comparable to that shown in other studies. For example, up to 37% of the no-contact control group in the study by Ball and colleagues

(2002) showed increases on a range of cognitive measures despite not receiving a cognitive training program. That 55.7% of the control group in the present study displayed an increase in CR may reflect unreported involvement in mentally complex and stimulating activities outside of the THBP. It would have been informative to have an ongoing measure of non-educational life experiences and activities, beyond baseline, in order to explain control group growth.

For three of the groups, prior CR tended to be associated with higher current CR at baseline. This finding suggests that prior life experience, such as education, promotes higher levels of CR in later life. However, in the intervention-maintainers group, prior CR was associated with lower current CR at baseline. Due to the small sample size of this group (n = 15) such associations must be treated with caution. There was no association between prior CR and the rate of linear or quadratic change over time. Thus, prior CR predicts initial levels of current CR for the majority of participants, but is not predictive of the rate or degree of change in CR that occurs following exposure to university level education.

Key limitations of the current study are the sample selection method and the lack of randomisation to groups (e.g. the experimental group or the control group). The volunteer based sample may have led to a self-selection bias, whereby the experimental participants involved in the THBP are likely to place greater interest and value in further education than the wider community. However, this sample bias does not discredit the study because the THBP is designed to determine whether increased mental activity in later life is beneficial to various cognitive functions in an aging population. As such, the THBP has utilised higher education as the vehicle for complex mental activity. The finding of increased cognitive reserve is evidence of an effect of increased mental activity that could be achieved through

the pursuit of mentally stimulating activities other than higher level education, such as more informal community adult education programs. Further, the lack of randomisation to groups indicates that an inherent limitation of the study is that baseline variables may have influence the results. While care was taken to statistically examine whether baseline variables such as age and education and control for this influence, the naturalistic nature of the design is advantageous in the sense that it creates high external validity.

Another point worthy of discussion is how missing values were treated, and consequently, how this process could impact the results obtained. Missing data were assumed to be missing at random, and maximum likelihood with robust standard errors estimation was used to integrate all available information based on this missing at random assumption. However, the efficiency of this technique is limited by the amount and type of missing data (Nagin & Odgers, 2010). Though at this early stage of the THBP, attrition was relatively low, in future it will be important to analyse whether rates of attrition correlate with CR and other outcome variables. As (Nagin & Odgers, 2010) points out, this process will show whether missing values across occasions are systematic or un-systemic, and if necessary attempts can then be made to address the non-ignorable missing data.

Though the benefit of early life education on late life cognitive function is well reported (Anstey & Christensen, 2000; Lenehan et al., 2015) this research is the first to investigate the potential benefit of a period of formal education in later-life to enhance CR. It also utilises a multivariate estimation of both pre-existing and current CR in order to provide an accurate evaluation of the potential benefit associated with the education intervention (Ward, Summers, Saunders, & Vickers, 2014). However, it is important to note that the modelling approaches utilised rely on extrapolation from an incomplete dataset. The THBP is an

ongoing study and it will be interesting to see whether these findings are robust once the full sample proceeds through all of the time points in future years. There are a number of limitations that should be noted in interpreting the results of the present study. Noticeably, the control group just reaches the minimum sample size of 100,which is typically preferred for latent growth modelling (Curran et al., 2010). The total number of person-by-time observations influences statistical power (Curran et al., 2010). Additionally, due to the progressive recruitment of participants into the THBP over a 4-year period, the models estimated are based on extrapolation from an incomplete dataset, where some individuals have only one or two observations over time. This may result in increased within group variability, as indicated by a larger standard error of the mean, which is more evident in the control group and therefore less power to detect significant intercept and slopes. Future research will re-examine the findings of the present analysis as the complete THBP participant pool completes assessment over all time points.

Although unavoidable due to the design of the present study, it is also important to note that the recruitment of voluntary participants into the THBP may result in a self-selection bias of older adults with an interest in pursuing further education and a history of higher level secondary school education required for entry into University level study. Therefore the participants in the THBP are likely to have a higher level of prior education and a greater interest in education than the wider community. However, it is important to note that the THBP is designed to determine whether increased mental activity in later life is beneficial to cognitive function in an aging population. As such, the THBP has utilised higher education as the tool for stimulating mental activity. A finding of increased cognitive capacity would be evidence of an effect of increased mental activity that could be achieved through the pursuit of mentally stimulating activities distinct from university level education.

To summarise, the findings of the present study indicate that engaging healthy older adults in university level education of a minimum of 12 months results in a measureable and significant increase in cognitive reserve. Future research is planned to determine whether this increase in cognitive reserve is sufficient to offset age-related cognitive decline and further, whether this increase in CR mitigates the risk for degenerative conditions such as dementia, or delays the onset of clinical symptoms of dementia in those at risk of dementia.

Chapter 5

Does enhancing cognitive reserve in older adults through further education lead to improved cognitive function: The Tasmanian Healthy Brain Project

Abstract

Background: The strong link between education and cognitive performance suggests that a period of education in later-life could enhance cognitive function. This is suggested as a non-pharmacological approach to reduce age-related cognitive decline and protect against AD.

Methods: Changes in episodic memory, working memory, executive function and language processing in older adults participating over 4 years in the Tasmanian Healthy Brain Project were examined. The annual cognitive performance of 459 participants enrolled in the THBP were examined. We compared a previously identified group of participants who undertook university study and demonstrated increased CR (intervention) against a group who did not engage in further education and did not display a change in CR (control).

Results: Multiple group latent growth curve modelling revealed no significant group difference in the trajectory of scores in episodic memory, working memory, or executive function between the two groups. However, the intervention group displayed significantly better performance at baseline relative to controls for language processing. Further, the intervention group displayed a significant improvement in language processing over time, with the control group remaining stable.

Conclusions: In a group of older adults with improved cognitive reserve resulting from attending university, we found that there is a commensurate improvement in language processing capacity over time but not episodic memory, working memory or executive function, within the first 4 years of the study. These results suggest that, in the short term, complex mental stimulation results in improved cognitive reserve and crystallised cognitive function but does not result in improvements to more fluid cognitive functions. It remains possible that over extended period of time differences may emerge in these other cognitive domains reflecting a decrease in the rate of age-related cognitive.

Introduction

Interventions designed to enhance cognitive function are a promising non-pharmacological approach to delaying and preventing Alzheimer's disease. The positive benefits of such interventions presumably occur due to an increase in cognitive reserve (CR). CR is the proposed mechanism through which pathology related cognitive deficits can be delayed or reduced (Stern, 2009). This occurs through a capacity to utilise pre-existing brain networks efficiently (neural reserve) or to enlist alternate brain networks (neural compensation) such that cognitive function can be preserved despite neural degeneration (Stern, 2002).

Education, occupational attainment, and leisure activities have been shown to make independent contributions to CR (Foubert-Samier et al., 2012). Consequently, recent research has sought to provide a multidimensional measure of CR (e.g. Bonner-Jackson et al., 2013; Serra et al., 2015; Ward, Summers, Saunders, & Vickers, 2014) in order to assess the relationship between CR and cognitive functioning in the presence of brain pathology. In a sample of individuals with prodromal Huntington disease, Bonner-Jackson et al. (2013) found higher levels of reserve to be associated with a reduced rate of decline in executive function over time. It has also been shown that patients with high CR sustain a higher degree of brain damage before the same level of clinical symptoms is expressed in patients low in CR (Serra et al., 2015). Interestingly, the same study highlighted the possibility that CR does not influence cognitive performance in healthy older adults, nor during the advanced stages of AD neuropathology (Serra et al., 2015). Rather, CR might act as a buffer between cognitive function and brain pathology in only the early stages of AD (Serra et al., 2015). Nevertheless, findings point to the importance of researching interventions which enhance CR in later-life.

The findings from a number of studies infer that CR can be enhanced or modified through environmental and lifestyle factors. Randomised control trials report improvements in executive control (Kramer et al., 1999), cognitive flexibility and mental speed (Masley et al., 2009), and episodic memory function (Ruscheweyh et al., 2011) following an aerobic activity intervention. Cognitive training programs have shown success in improving memory (Ball et al., 2002; Envig et al., 2010; Kirchhoff et al., 2012; Willis et al., 2006), generalised memory and attention (Smith et al., 2009) reasoning (Ball et al., 2002; Willis et al., 2006) and speed of processing (Ball et al., 2002; Willis et al., 2006). Although there is some evidence to suggest that cognitive training is generalizable to other cognitive domains (Smith et al., 2009), overall the literature points to specific rather than generalised benefits of training. Owen et al. (2010) report improvement in specific cognitive functions following computerised cognitive training, but found no evidence of transfer or generalisation of specific cognitive improvements to other specific cognitive functions or to general cognitive ability. Nonetheless, it is possible that improvements in cognitive function arising from cognitive training may be secondary to an underlying training induced enhancement of CR.

Another potentially modifiable lifestyle factor receiving much research attention is the role of education in reducing age-related cognitive decline. Education is thought to increase CR through the growth of new cognitive strategies (Manly et al., 2004). Educational attainment has been shown to decrease risk of dementia (Valenzuela & Sachdev 2006) and moderate the relationship between brain pathology and neuropsychological test performance in memory, language, speed of processing and visuospatial skills (Bennett et al., 2003b; Dufouil et al., 2003; Rentz et al., 2010). Education during childhood and early adulthood may also be implicated in cognitive performance and rates of normal age-related cognitive decline later in life. As a part of the normal aging process, cognitive functions including working memory,

episodic memory, processing speed, visuospatial skills and executive function, decline rapidly in later life (Salthouse, 2010b). A small number of studies have found high levels of education to be associated with a reduced rate of decline in information processing speed (Bosma et al., 2003), memory (Bosma et al., 2003; Cullum et al., 2000) and general mental status (Alley et al., 2007; Bosma et al., 2003).

A larger body of research, however, negates this relationship, reporting the rate of decline is constant regardless of level of early life education. This finding has been demonstrated across a range of cognitive functions including memory (Der et al., 2010; Proust-Lima et al., 2008; Van Dijk et al., 2008), processing speed (Christensen et al., 2001; Zahodne et al., 2011), language processing (Seeman et al., 2005; Tucker-Drob et al., 2009; Van Dijk et al., 2008) and visuospatial skills (Cullum et al., 2000; Seeman et al., 2005). Despite this, research consistently shows that higher levels of education in early adulthood are associated with superior performance on measures of cognitive function (Anstey & Christensen, 2000; Lenehan et al., 2015). Consequently, whether or not education moderates the rate of normal age-related cognitive decline, enhancing an individual's level of cognitive function through education could help to preserve normal cognitive function for a longer period of time in the presence of neurodegeneration.

Regardless of the success of cognitive training program interventions, and the strong link between education and cognitive performance, the potential benefit of an education based intervention in later life has not been directly examined. The main objective of the THBP is to determine the capacity of university-level education to enhance cognitive reserve in healthy older adults and subsequently reduce age-related cognitive decline and risk for neurodegenerative disease (Summers et al., 2013). We have demonstrated that further

education leads to a measurable increase in current CR in the intervention group (Lenehan et al., 2015, Under Review). The aim of the present paper is to examine whether the increase in CR observed in the intervention group is associated with a change in neuropsychological test performance and whether there is a decreased rate of cognitive decline over time in the intervention group relative to the control group. It was hypothesised that those receiving the education based intervention would have a reduced rate of decline observed across multiple cognitive domains relative to healthy control subjects.

Method

Participants

The Tasmanian Healthy Brain Project (THBP) (Summers et al., 2013) is a prospective longitudinal study of older adults engaging in university level education. The THBP sample has been recruited progressively from 2011-2014. Data analysed in the present paper is collected from 459 adults aged between 50 and 79 years who had participated in the THBP as of the 31 December 2014. Those in the intervention group (n = 359) had undertaken a minimum of 12 months part-time or full-time university study, with a minimum study load of two units at undergraduate or post graduate levels. The remaining 100 participants were a control reference group. These individuals did not take part in any tertiary level study. However, previous analysis of the control and intervention groups revealed two subclasses of individuals within each group (Lenehan et al., 2015, Under Review). To briefly summarise, CR theory posits that any improvement in cognitive function seen for the intervention group would be caused as a result of a positive effect of further education on CR. As such, we examined whether the intervention group displayed increased CR relative to the control group

over the first 4 years of the THBP. Growth Mixture Modelling (GMM) revealed two latent classes of participants within the control and the intervention groups, based on patterns of performance in current CR over time. In the control group, 55.7% of participants displayed improved CR, with the remaining 43.3% of participants displaying stable CR. The cognitive domain scores (see analysis below) over time of these two classes were compared using a series of repeated measures analysis of variance (ANOVA). This revealed no significant differences in cognitive domain scores over time and consequently all 100 participants were retained and collapsed into a single control group for the current study. The majority of the intervention group displayed increased CR over time (92.5%), while the remainder displayed no change in CR (stable, 7.5%). A series of repeated measures ANOVAs revealed significant differences in cognitive performance between the two classes. As there were insufficient numbers in the stable CR class (n = 15) to analyse the group separately, these 15 people were excluded from the present analysis. Thus, in the present paper we examined the cognitive performance of two groups, a control group (n = 100) who did not undertake further education, and an intervention group (n = 344) who undertook university level education and have demonstrated a significant increase in CR over the first 4 years of the THBP (Lenehan et al., 2015, Under Review).

Participants who presented with a medical, neurological, or psychiatric disorder that could potentially impair cognition were precluded from entry into the THBP. The project was approved by the Human Research Ethics Committee (Tasmania) Network and further details of the study protocol have been previously published (see Summers et al., 2013).

Materials

Participants in the THBP completed a comprehensive testing battery. For detailed project protocols refer to Summers et al. (2013). The Dementia Rating Scale, 2nd edition (DRS-2; Jurica et al., 2001); the Hospital Anxiety and Depression Scale (HADS; Snaith, 2003) and the Medical Health Status questionnaire (Summers et al., 2013) were administered to ensure participants were free from dementia and of sound psychological and physical health. Estimates of pre-morbid intellectual capacity were obtained using the Wechsler Test of Adult Reading (WTAR; The Psychological Corporation, 2001).

Neuropsychological performance.

The neuropsychological test battery comprised 14 tests encompassing four broad cognitive domains: episodic memory, working memory, executive function and language processing. Composite scores were created for each cognitive domain by Principal Components Analysis consistent with an approach utilised in previous work by this group (Ward, Summers, Saunders, Janssen, et al., 2014). Briefly, the episodic memory score comprised Logical Memory test (LMI, LMII; Wechsler, 1997b), Rey Auditory Verbal Learning Test (RAVLT; Lezak et al., 2012) and Paired Associates Learning (PAL; Cambridge Cognition Limited, 2012). The working memory score comprised Digit Span (Wechsler, 1997a), Letter Number Sequencing (Wechsler, 1997a), Spatial Span (SSP; Cambridge Cognition Limited, 2012) and Spatial Working Memory (SWM; Cambridge Cognition Limited, 2012), the Executive Function score comprised Trail Making Test Trail B (TMT-B; Strauss et al., 2006), 24-item Victoria version Stroop Colour-Word Test (Stroop C; Strauss et al., 2006) and Rapid Visual Processing (RVP A'; Cambridge Cognition Limited, 2012). Finally, language processing

score comprised Vocabulary (Wechsler, 1997a), Comprehension (Wechsler, 1997a) and Boston Naming Test (BNT; Kaplan, Goodglass, & Weintraub, 1983). For each respective test, individual raw scores were standardised to z-scores against the sample mean and standard deviation at baseline assessment. Therefore, an individual's performance on each neuropsychological test over time is referenced against their performance at baseline in standard deviation units above or below the 50^{th} percentile. To create the domain composite scores, the z-scores from relevant tests were multiplied by the factor coefficients produced from the principal components analyses (PCA). To this effect, cognitive domain composite scores represent decline or improvement over time relative to the sample mean at baseline.

Procedure

After obtaining consent the elements of the full THBP test battery used in the present analysis were administered to each participant in the following order: WTAR, DRS-2, Medical Health Questionnaire, PAL, RAVLT, Logical Memory I, SSP, Digit Span, SWM, Letter Number Sequencing, Logical Memory II, Vocabulary, Comprehension, BNT, RVP A', STROOP C, TMT B, HADS. An approximate 20 minute delay occurred between the administration of LMI and LMII. The full THBP assessment battery took approximately four hours to complete and subjects were encouraged to take short breaks as needed to avoid fatigue (Summers et al., 2013). Participants were reassessed at one year intervals (± one month).

Analysis

Principal components analyses.

Initially, four separate PCAs were conducted to compute composite scores for each cognitive domain at baseline using SPSS, version 19. PCA was selected in order to reduce the number of variables while retaining as much of the original variance as possible (Conway & Huffcutt, 2003). Previous studies from this group have used similarly constructed composite scores (Ward, Summers, Saunders, & Vickers, 2014).The factorability of items in each cognitive domain was assessed with reference to a number of recognised criteria. Firstly, it was observed that all tests specific to each domain correlated at least .3 with at least one other test. Secondly, the Kaiser-Meyer-Olkin measure of sampling adequacy was above the recommended value of .60 (Hair, Anderson, Tatham, & Black, 1998) and in each case Bartlett's test of sphericity was significant. The diagonals of the anti-image correlation matrices (measures of sampling adequacy) were all above the .5 recommended minimum (Field, 2009). Based on these indicators, factor analysis was considered to be suitable with all 14 neuropsychological tests.

It was specified in the analysis that one component be extracted for each domain of cognitive function. Given the large sample size, item factor loadings of ≥ .3 could be considered statistically significant (Hair et al., 1998). However, only factor loadings of ≥ .4 were considered to have practical interpretability in the present study. The results of the PCA are presented in Table 7. Factor coefficients for each of the test scores were combined into a single factor score using a regression method, yielding a z-score. The equation that resulted in episodic memory score = .356 (LM I) + .346 (LM II) + .305 (RAVLT) + .245 (PAL). The equation that resulted in working memory score = .397 (Letter Number Sequencing) + .376

(Digit Span) + .325 (SWM) - .306 (SSP). The equation that resulted in executive function score = .439 (Stroop C) + .424 (TMT B) - .460 (RVP A'). Finally, the equation that resulted in language processing score = .360 (Boston Naming Test) + .442 (Comprehension) + .477 (Vocabulary). To calculate domain composite scores for the subsequent time points (T1, T2, T3), baseline referenced z-scores for the relevant tests were imputed into these formula. Baseline referenced z-scores were calculated because they indicate whether the individual has improved or decline since their start point have been used in other published longitudinal studies of age-related cognitive decline (e.g. Zahodne et al., 2011).

Table 7: Principal component analysis results for composite cognitive domain scores.

Cognitive domain	Eigenvalue (variance explained)	Test Name	Mean	SD	Loading
Episodic memory	2.51 (62.65%)	LM I immediate recall total	48.31	8.30	.89
		LM II delayed recall total	30.15	6.41	.87
		RAVLT 1-5 recall total	53.14	8.86	.76
		PAL first trial memory score	18.35	3.35	.61
Working memory	2.01 (50.23%)	Letter number sequencing	11.67	2.39	.80
		Digit span	18.77	3.91	.76
		SSP span length	5.76	1.20	.65
		SWM between errors	25.63	18.58	-.61
Executive function	1.71 (57.03%)	RVP A'	.91	.05	-.79
		Stroop C time	25.94	7.53	.75
		TMT B time	59.02	19.67	.73
Language processing	1.81 (60.35%)	Vocabulary	56.90	5.78	.86
		Comprehension	26.15	3.41	.80
		Boston Naming Test	57.68	2.90	.65

Multiple group latent growth curve modelling.

Multiple group latent growth curve modelling (LGCM) was conducted using Mplus 7.0 (Muthén & Muthén, 1998-2012) maximum likelihood estimation. Multiple group LCGA was conducted because it enables the direct comparison of the control and intervention group, for example whether the slope of the intervention group significantly departs from slope of the control group which is important in analysing the effect of the intervention. A more standard independent analysis of each group separately would not enable this essential comparison.

Initially, the control group and the intervention group were examined separately to check that both groups had the same basic trajectories (i.e. linear or quadratic). Then approach outlined by (Acock, 2005) was followed. A model was estimated simultaneously for the control and the intervention groups with no constraints on any parameters. This allowed the estimated parameters of the model to be different in terms of: the intercept, the slope term and variances. To then test whether the intercept and slopes were significantly different between the control and intervention groups, two constrained models were estimated. One with the intercept term held equal across groups to test whether the groups had a different intercept. The second model held the linear term equal across groups to test whether the groups had a different slope. A series of chi-square difference tests then revealed if the model which allowed intercept and slope parameters to vary between groups was a significantly better fit compared to the constrained models.

In all models, time was paramatised with time scores that represented years since study entry and the intercept loadings of the four time points were fixed at one. Initially, Mplus default

parameters were used which were as follows: the means, variances and covariances of the growth factors were estimated. Incremental model changes such as fixing growth factor variance to zero were also investigated to find the best fitting model. In each model, the intercept term represented the mean of each respective cognitive domain score, the linear growth term represented the annual rate of change in score, and the quadratic growth term indicated the change in the rate of change (accelerating or decelerating change).

Model fit

A number of statistics were considered in deciding whether a model was a good representation of the data. The likelihood-ratio chi-square is a popular statistic used to assess overall fit. In general a smaller, insignificant value at the level of .05 indicates a well-fitting model (Hooper, Coughlan, & Mullen, 2008). However, because chi-square is sensitive to sample size, the statistic can be prone to type II error in the case of large sample sizes (Hooper et al., 2008) and consequently a range of other fit indices were considered. The root mean squared error of approximation (RMSEA) is a measure of closeness of fit with values of < .7 indicating good fit and < .03 indicating excellent fit (Steiger, 2007). An RMSEA value of ≥ .8 is considered a poor fit. Finally, comparative fit index (CFI) was also considered with values of ≥ .95 indicative of good fit (Hu & Bentler, 1999).

Results

Descriptive Data

The sample consisted of 444 older adults, aged between 50 – 79 years at baseline. Overall, the sample was of above average intelligence, free from dementia, and not clinically depressed or anxious (see Table 8). Males were under-represented in the sample (32%). This is a common feature of longitudinal research in this field (Zahodne et al., 2011), and was a characteristic of both control and intervention groups in the current study. A breakdown of demographic information for each group is presented in Table 8. The intervention group was approximately two years younger than the control group at baseline ($t(442) = 3.84$, $p. < .001$) and had approximately one additional year of prior education ($t(442) = -2.45$, $p. < .05$). However, as there were no significant correlations between age or education and

neuropsychological performance across any of the four time points, the decision was made not to include these factors as covariates in further analyses. Cohen's (1988) cut off values were utilised with only correlations of a moderate (≥ .5) or large (≥ .8) magnitude considered meaningful given the large sample size. In addition, including age and baseline education in the model did not significantly improve the fit of the model, or dramatically alter the structure of the model, consequently the decision was made not to include age as a covariate in the analyses. There were no significant differences between the control and intervention groups across baseline measures of global cognition, estimated premorbid IQ, level of anxiety or level of depression. Means and standard deviations for cognitive domain scores at each time point as a function of group are presented in Table 9.

Table 8: Sample demographic information as a function of group.

	Control *N* at T0 = 100 M *(SD)*	Intervention *N* at T0 = 344	*Independent samples t-test* *p.*
Female *N* (%)	63 (63%)	238 (69.2%)	(χ^2) = .24
Baseline Age	62.49 *(6.24)*	59.59 *(6.77)*	< .001
Prior Education	13.53 (2.65)	14.28 (2.69)	< .05
DRS-2 AEMSS	11.91 *(2.27)*	11.93 *(2.10)*	.94
WTAR (est. FSIQ)	112.49 *(5.05)*	112.56 *(5.47)*	.91
HADS - Anxiety	5.51 *(2.91)*	5.24 *(3.14)*	.44
HADS - Depression	2.82 *(2.32)*	2.42 *(2.27)*	.13

DRS-2 AEMSS = Mattis Dementia Rating Scale age and education corrected Mayo scaled score; WTAR (est FSIQ) = Wechsler Test of Adult Reading Scale estimated full scale IQ; HADS = Hospital Anxiety and Depression Scale; CR = cognitive reserve.

Table 9: Sample neuropsychological performance as a function of group.

		Control			Intervention		
		N	M	*(SD)*	*N*	M	*(SD)*
Episodic Memory	T0 -Baseline	100	-.15	1.01	343	.04	1.00
	T1	91	-.07	.95	272	.13	.99
	T2	66	.16	.89	199	.42	.97
	T3	46	.39	.96	102	.79	.90
Working Memory	T0 -Baseline	100	-.13	1.03	342	.03	1.00
	T1	91	-.08	1.01	271	.03	.99
	T2	67	.02	.98	200	.094	.98
	T3	46	-.02	1.15	102	.21	1.05
Executive Function	T0 -Baseline	100	-.03	.61	342	.02	.62
	T1	91	.03	.64	270	-.03	.65
	T2	67	-.12	.62	198	-.10	1.10
	T3	45	-.14	.59	101	.02	.65
Language Processing	T0 -Baseline	100	-.12	1.03	344	.07	.96
	T1	92	-.04	1.02	272	.19	.95
	T2	68	-.08	1.24	201	.35	.83
	T3	46	.02	.90	102	.29	.87

Table 10: Fit indices of separate group analysis latent growth curve modelling.

			Chi square test							Δχ2 difference
Cognitive domain		Group	N	χ2	df	p	RMSEA (CI)	SRMR	CFI	*p.*
Episodic memory	Control	Linear	100	3.18	7	.87	< .001, (.00 -.06)	.04	1.00	NS
		Quadratic	100	1.64	6	.95	< .001 (.00-.01)	.03	1.00	
	Intervention	Linear	344	18.52	7	.01	.07 (.03- .11)	.04	.98	< .01
		Quadratic	344	10.46	6	.11	.05 (.00-.09)	.03	.99	
Working memory	Control	Linear	100	8.62	7	.28	.05 (.00-.14)	.07	.99	NS
		Quadratic	100	8.55	6	.20	.07 (.00-.16)	.07	.99	
	Intervention	Linear	343	5.53	7	.60	< .001 (.00-.06)	.02	1.00	NS
		Quadratic	343	5.52	6	.48	< .001 (.00-.07)	.02	1.00	
Executive function	Control	Linear	100	4.01	7	.78	< .001 (.00-.83)	.04	1.00	NS
		Quadratic	100	3.42	6	.75	< .001 (.00-.09)	.04	1.00	
	Intervention	Linear	343	3.37	7	.85	< .001 (.00-.04)	.04	1.00	NS
		Quadratic	343	2.51	6	.87	< .001 (.00-.04)	.04	1.00	
Language Processing	Control	Linear	100	13.48	7	.06	.10 (.00-.17)	.11	.97	NS
		Quadratic	100	12.68	6	.05	.11 (.01-.19)	.11	.97	
	Intervention	Linear	344	14.76	7	.04	.06 (.01-.10)	.07	.98	NS
		Quadratic	344	11.28	6	.08	.05 (.00-.10)	.06	.99	

Episodic Memory

In the control group both the linear and the quadratic models were a good fit of the data (Table 10). The chi-square difference test indicated the quadratic model did not provide a significantly better fit of the data. In the intervention group however, the quadratic model was a significantly better fit of the data. For the purpose of the multiple group analysis the decision was made to fit the linear model to both groups, rather than potentially over fitting a quadratic model to the control group. In both groups, the linear models were initially inadmissible due to negative variances on the linear growth factor. As this variance was small and not significant the variance of this term was fixed at zero which solved the problem.

The linear model was fit simultaneously to both groups, with the variance in the linear growth factor fixed at zero. The model was a good fit of the data ($\chi^2_{(14,\ N=\ 444)} = 21.70$, $p. = .09$, RMSEA = .05, CFI = .99). In both groups the intercept was not significantly different from zero and the linear term was positive and significant, indicating improvement in episodic memory score over time (Table 11 and Figure 8). The rate in improvement is faster in the intervention group compared to the control group. To examine whether the intercept and linear growth terms were significantly different between the control group and the intervention group two chi-square difference tests were conducted.

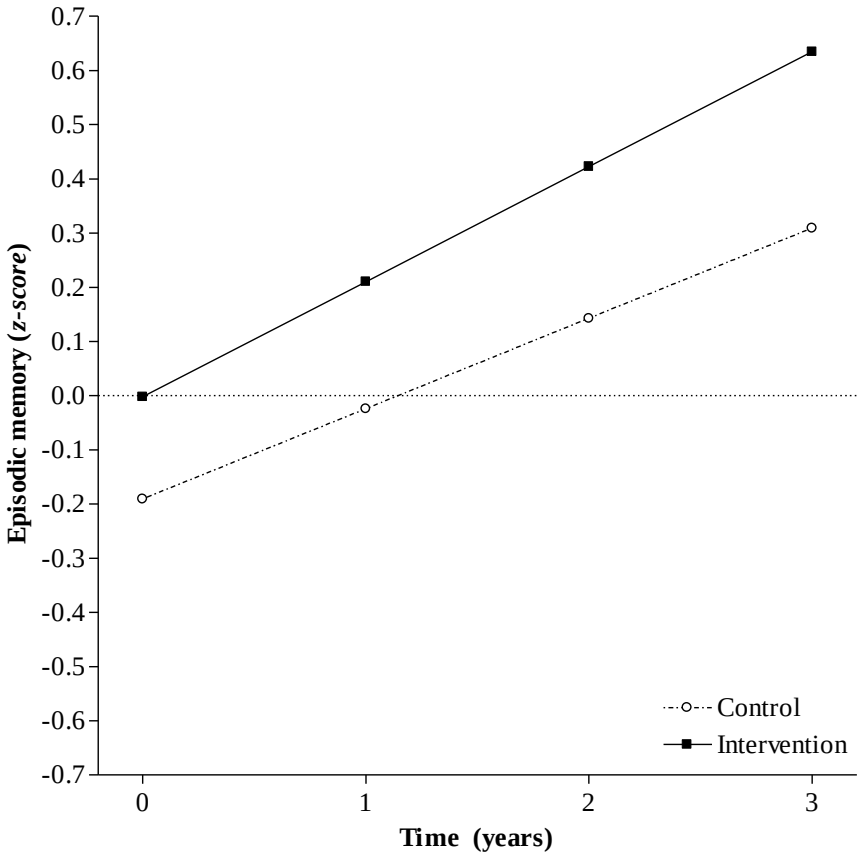

Figure 8: Model-predicted episodic memory trajectories over 4 years for individuals in the control group and the intervention group.

A chi-square difference test between the freely estimated model and a constrained model in which the intercept term was held equal across groups revealed that baseline performance was not significantly different between the two groups ($\Delta\chi^2_{(1)} = 2.93$; *p.* = > .05). The second chi-square difference test was to compare the freely estimated model and a constrained model in which the linear growth term was held equal across groups and revealed no group differences in the rate of increase in episodic memory score ($\Delta\chi^2_{(1)} = 1.91$; *p* = > .05).

Working Memory

For both groups the linear and quadratic models both provided adequate fit of the working memory data (Table 10). Considering that the quadratic models were not a significantly better fit compared to the linear model in either the control group or the intervention group analysis, the decision was made to retain the linear trajectory for the purpose of the multiple group analysis. Again, negative variances in the linear growth term resulted in fixing the variance to zero for this factor. The estimated simultaneous model fit the data well ($\chi^2_{(14, N= 443)} = 14.14$, *p*. = .44, RMSEA = .01, CFI = 1.00). Neither group had an intercept significantly different from zero. Though the linear growth term was positive for each group, only the intervention group had a slope significantly different from zero (Table 11 and Figure 9).

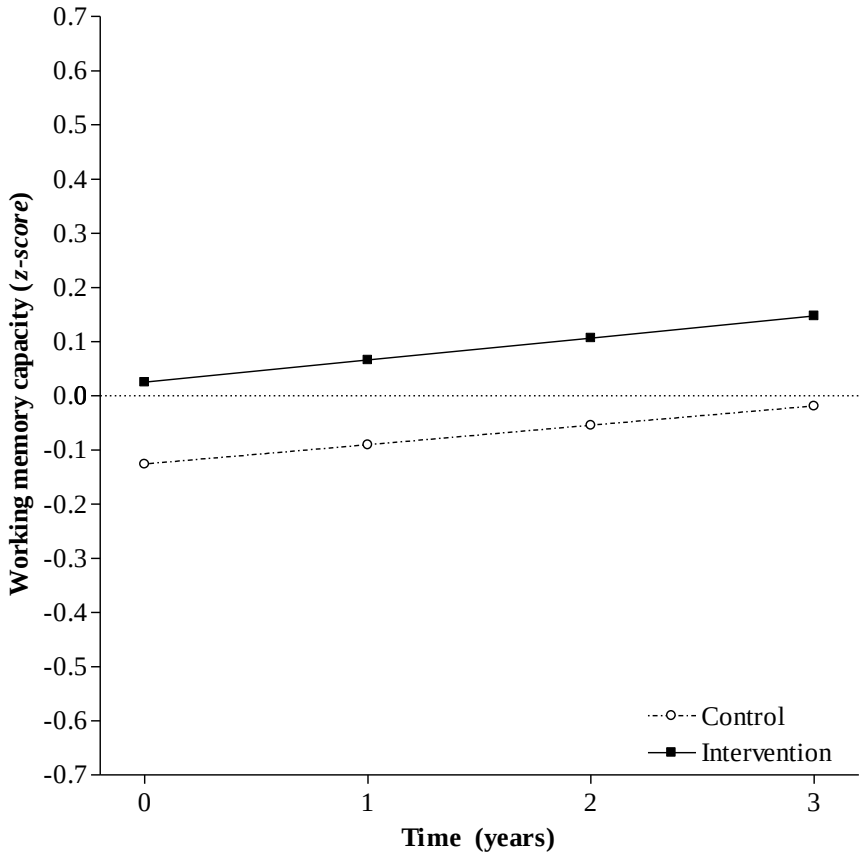

Figure 9: Model-predicted working memory trajectories over 4 years for individuals in the control group and the intervention group.

Consistent with the findings in episodic memory, the chi-square difference tests revealed that there were no significant differences between the two groups in terms of the intercept term ($\Delta\chi^2_{(1)} = 1.75$; *p.* = > .05), or the linear growth term ($\Delta\chi^2_{(1)} = .03$; *p* = > .05).This indicates that groups did not have a different intercept or rate of change in working memory score over time.

Executive Function

A linear model was a good fit of the data for each group (Table 10), though it was necessary to fix the variance of the linear growth factor to zero to avoid negative variance. Adding a quadratic growth term did not significantly improve model fit and as such the linear model was retained for the purpose of the multiple group analysis.

When fit simultaneously to both groups the linear model was a good fit of the data, ($\chi^2_{(14, N= 444)} = 7.39$, *p.* = .92, RMSEA = .00, CFI = 1.00). Neither group had an intercept significantly different from zero (Table 11). The linear growth term was negative in both groups indicating a downward trend though this was not significant (Table 11 and Figure 10). Consistent with the findings in memory, the chi-square difference tests revealed that there were no significant differences between the two groups in terms of the intercept term ($\Delta\chi^2_{(1)} = .02$; *p.* = > .05), or the linear growth term ($\Delta\chi^2_{(1)} = .14$; *p* = > .05).This indicates that groups did not differ significantly on baseline score or rate of change in executive function over time.

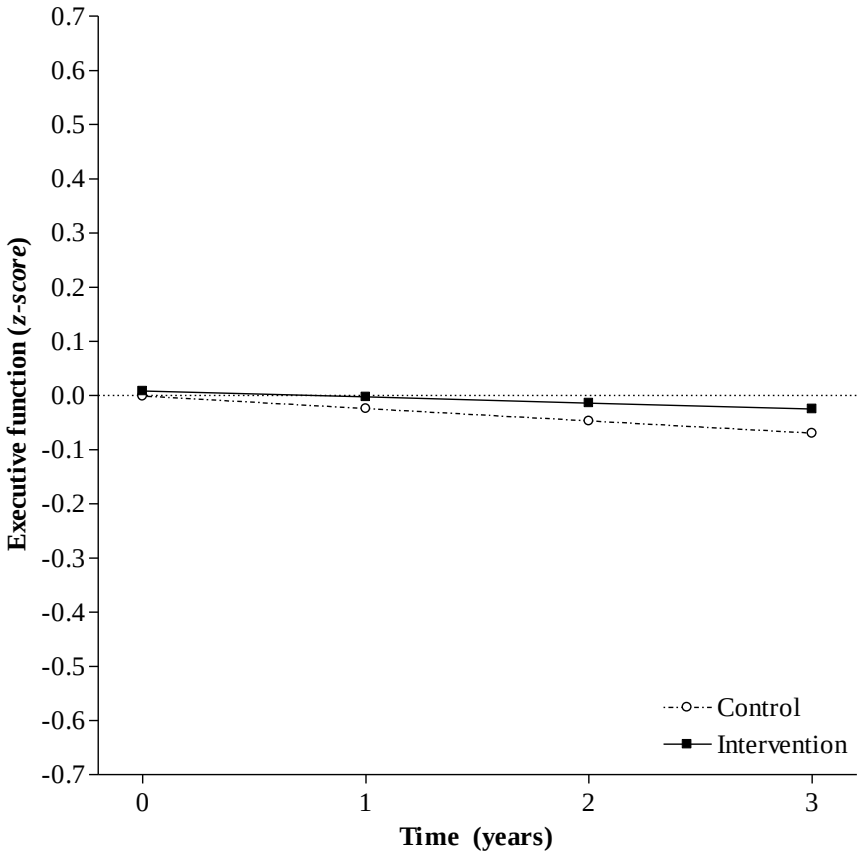

Figure 10: Model-predicted executive function trajectories over 4 years for individuals in the control group and the intervention group.

Language Processing

A linear model provided adequate fit of the data for each group (Table 10), though it was necessary to fix the variance of the linear growth factor to zero to avoid an inadmissible model. Adding a quadratic growth term did not significantly improve model fit and as such the linear model was retained for the purpose of the multiple group analysis. The poorer fit of the model in the control group relative to the intervention group could reflect the greater variance in scores inherent with smaller sample sizes.

When fit simultaneously to both groups the linear model fit reasonably well, ($\chi^2_{(14, N= 444)}$ = 28.23, *p.* = .01, RMSEA = .07, CFI = .98). Neither group had an intercept significantly different from zero. The linear growth term was negative in the control group indicating a downward trend though this was not significant (Table 11 and Figure 11). In the intervention group the linear term was positive and significant, suggesting an improvement in language processing score over the four years (Table 11). A chi-square difference test revealed that the model with freely estimated intercepts was a significantly better fit compared to the constrained model in which intercepts were held equal across groups ($\Delta\chi^2_{(1)}$ = 6.46; *p.* = < .05). This indicates that the intervention group had a significantly higher score at baseline compared to the control group. A chi-square difference test between the freely estimated model and a model in which the linear term was held equal across groups was also significant, ($\Delta\chi^2_{(1)}$ = 10.41; *p.* = < .01), indicating a significant difference in the rate of change between the two groups with the intervention group increasing score and the control group remaining stable.

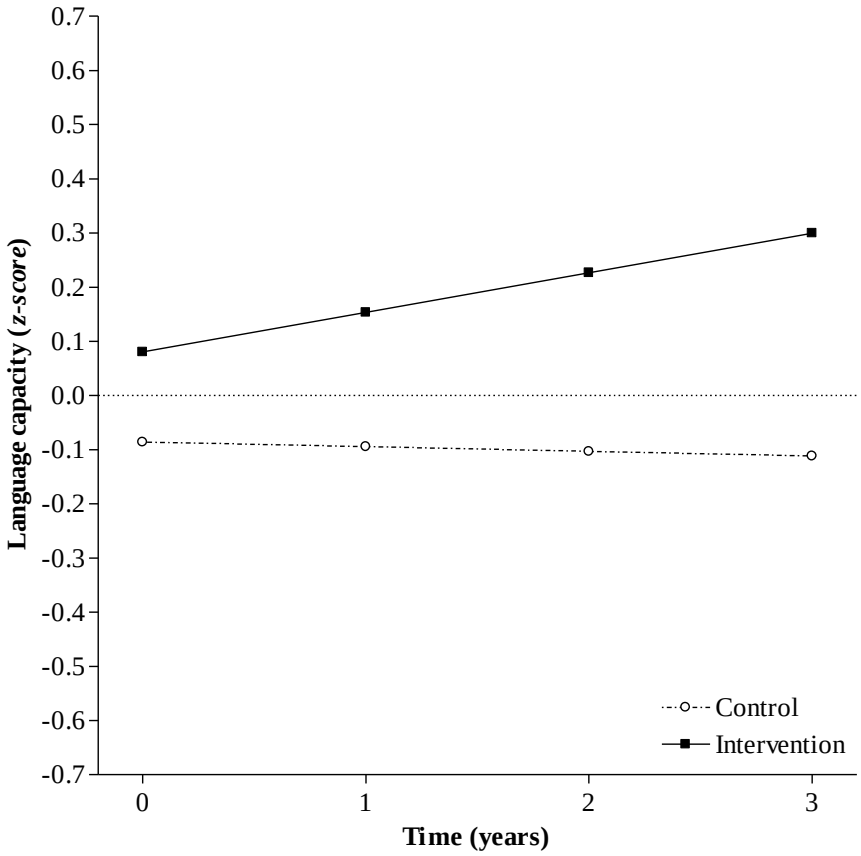

Figure 11: Model-predicted language processing trajectories over 4 years for individuals in the control group and the intervention group.

Table 11: Estimates (SE) of group specific means for latent variables.

	Control	**Intervention**
	Model estimates (*SE*) (*N* =100)	**Model estimates (*SE*) (*N* = 344)**
Episodic memory		
Intercept	-.19 *(1.00)*	-.002 *(.05)*
Variance	.73 *(.11)*	.78 *(.07)***
Linear growth rate	.17 *(.03)**	.212 *(.02)***
Variance	.00 *(.00)*	.00 *(.00)*
Working memory		
Intercept	-.126 *(.10)*	.025 *(.05)*
Variance	.87 *(.13)***	.80 *(.07)***
Linear growth rate	.036 *(.03)*	.041 *(.02)**
Variance	.00 *(.00)*	.00 (.00)
Executive function		
Intercept	-.001 *(.06)*	.008 *(.032)*
Variance	.15 *(.03)***	.19 *(.02)***
Linear growth rate	-.023 *(.027)*	-.011 *(.018)*
Variance	.00 *(.00)*	.00 *(.00)*
Language processing		
Intercept	-.107 *(.10)*	.059 *(.05)*
Variance	.84 *(.13)***	.57 *(.06)***
Linear growth rate	.072 *(.44)*	.170 *(.06)***
Variance	.00 *(.00)*	.00 *(.00)*

Note: * $p. < .05$, ** $p. < .01$.

Discussion

In the present study, we observed no significant decline in episodic memory, language processing, working memory, or executive function. Episodic memory performance

significantly increased in both groups, whereas only the intervention group demonstrated a significant improvement in working memory capacity. Importantly, there were no significant differences between the control and the intervention group in the rate of change over time in episodic memory, working memory, or executive function. For language processing capacity there were significant group differences in the intercept, with the intervention group performing higher at baseline compared to controls. In addition a significant difference in the rate of change in language capacity over time was detected between groups, with the intervention group displaying a significant linear increase in language processing capacity and the control group displaying no change in performance over the four year period.

The findings indicate that in a group of older adults who display a significant increase in CR subsequent to attending university, there is a measurable increase in language processing capacity not observed in a control reference group. The absence of a measureable increase in language processing capacity in the control group discounts the possibility that the increase observed in the intervention group is an artefact of familiarity or practice effects. The language processing measure, which comprised vocabulary and other acquired knowledge based tasks, can be considered to tap into crystallised knowledge. No such benefit was found for the fluid abilities reflected in the episodic memory or working memory domains of cognitive function. This suggests that the increase in CR observed in older adults undertaking university education intervention resulted in an increase in crystallised knowledge but not fluid abilities. It seems logical that in the context of university based education, an environment predicated on the acquisition of new information, that enhancement of crystallised, knowledge-based, cognitive functions such as language processing capacity would be observed.

The observed increase in language processing capacity over time may offer important protection against the ageing process. Lower levels of linguistic capacity in late life is associated with a greater rate of decline in general cognitive function as well as in specific cognitive functions including semantic memory, episodic memory, and spatial function (Farias et al., 2012). Lower levels of linguistic ability in early life has been shown to be associated with not only later-life cognitive impairments (Riley, Snowdon, Desrosiers, & Markesbery, 2005), but also to the presence of the hallmark characteristics of Alzheimer's dementia (Snowdon, Kemper, Greiner, Wekstein, & Markesbery, 1996). In the context of these findings, together with CR theory, the enhancement of language processing capacity may reduce the risk of dementia in the individual or reduce the functional impact of dementia in the presence of neuropathology. Crystallised knowledge, such as vocabulary, is one of the few cognitive functions that does not suffer substantial age-related cognitive decline (Hedden & Gabrieli, 2004). It is argued that this occurs due to continued vocabulary learning into later adulthood, through ongoing exposure to new words (Hartshorne & Germine, 2015). In comparison, fluid abilities such as episodic memory, reasoning, spatial skills and numeric ability show minimal change until after the age of 60 when decline begins and accelerates in the late 60's and early 70's (Hedden & Gabrieli, 2004). Considering that the majority of the participants in the THBP are currently early-mid 60 years of age and are therefore younger than the age at which age-related cognitive decline acceleration is reported to occur they are younger than the reported acceleration in age-related cognitive decline (ARCD) Additionally, many cognitive functions show minimal decline over a 5-10 year period (Hedden & Gabrieli, 2004). As such, the four year duration of the current study is likely of insufficient duration to detect a potentially subtle rate of decline. A longer observation period is required to ascertain if the expected rate of ARCD accelerates, becoming increasingly evident in fluid abilities, or whether this rate of decline is reduced or temporally delayed as a result of the observed

increase in CR triggered by undertaking university education in later life. It is not until an acceleration in ARCD is observed in the THBP sample that definitive conclusions can be drawn regarding whether the education intervention exerts a protective influence against ARCD and risk for neuro-degenerative diseases.

Longitudinal research investigating the role of early life education in ARCD utilising modelling approaches similar to that used in the present study fail to identify an association between level of early life education and the rate of decline across a range of measures of executive function, working memory, or episodic memory (e.g Der et al., 2010; Van Dijk et al., 2008; Zahodne et al., 2011). Yet the same studies consistently reveal an association between level of early life education and cognitive performance, reporting that individuals with higher levels of early life education continue to perform at a superior level of function over time in terms of general cognitive function and across specific domains (e.g Der et al., 2010; Van Dijk et al., 2008; Zahodne et al., 2011). Though such studies are not directly comparable to the present study, which is the first of its kind to adopt a long-term, prospective cohort approach to investigating the potentially positive role of later life education enhanced CR in older adults. These previous studies demonstrate that even if the rate of change does not differ substantially between those who received an intervention and those that did not, the improvement in CR previously identified in the THBP cohort (Lenehan et al., 2015, Under Review) may be sufficient to reduce the rate of ARCD over the medium to longer term and may exert a level of protection of cognitive function in the presence of neurodegeneration.

Though the benefit of early life education on late life cognitive function is well reported (Anstey & Christensen, 2000; Lenehan et al., 2015) this research is the first to investigate the

potential benefit of a period of formal education in later-life to enhance CR. Studying not only exercises the mind through learning new information, it also incorporates high-level social engagement which in itself contributes to enhanced cognitive function (e.g. Fratiglioni, Paillard-Borg, & Winblad, 2004; Seeman et al., 2001). The approach to intervention is highly naturalistic and the project offers a high level of external validity.

Key limitations of the current study are the sample selection and the lack of randomisation to groups (e.g. the experimental group or the control group). The volunteer based sample may have led to a self-selection bias, whereby the experimental participants involved in the THBP are likely to place greater interest and value in further education than the wider community. However, this sample bias does not discredit the study because the THBP is designed to determine whether increased mental activity in later life is beneficial to various cognitive functions in an aging population. As such, the THBP has utilised higher education as the vehicle for complex mental activity. The finding of increased language function in the experimental group is evidence of an effect of increased mental activity that could be achieved through the pursuit of mentally stimulating activities other than higher level education, such as more informal community adult education programs.

Further, the lack of randomisation to groups indicates that an inherent limitation of the study is that baseline variables may have influence the results. While care was taken to statistically examine whether baseline variables such as age and education and control for this influence, an advantage of the naturalistic nature of the design is that it has high external validity.

In conclusion, the present study examined whether improved CR in older adults resulting from undertaking university education in later life triggered a secondary improvement in

cognitive performance. The results indicated that while an improvement in language processing capacity was found, there was no change in episodic memory, or working memory, or executive function observed in those adults with late life university education relative to a control group within the time frame of the study to date. As data collection for THBP is ongoing, further examinations will be better positioned to draw conclusions at a later date.

Chapter 6

Does *APOE* allelic variation modify responsiveness to a tertiary education intervention designed to enhance cognitive reserve: The Tasmanian Healthy Brain Project

Abstract

Background: The strong link between education and cognitive performance suggests that a period of education in later-life could enhance cognitive function. However, little is known regarding whether apolipoprotein (*APOE*) allelic variation modifies an individual's responsiveness to an education intervention.

Methods: The annual cognitive performance of 444 healthy older adults, aged 50-70 years (M = 60.25, SD = 6.75) enrolled in the Tasmanian Healthy Brain Project was examined over a four year period. Episodic memory, working memory, executive function and language processing was assessed alongside *APOE* status (ε4 carriers and non-ε4 carriers). *APOE* data was used to conduct within and between group comparisons of cognitive function and the linear rate of cognitive change on a previously identified group of participants who undertook university study and demonstrated increased cognitive reserve (CR; intervention) and a group who did not engage in further education and did not display a change in CR (control).

Results: Multiple group Latent Growth Curve Modelling revealed no significant differences between intervention group ε4-carriers and non-ε4 carriers, or between control group ε4-carriers and non-ε4 carriers, either in baseline score or linear rate of change over time on any cognitive function. Additionally, the linear slope of the intervention ε4-carriers was not significantly different to the slope of non-ε4 carriers across any of the four cognitive domains.

Conclusions: We found no evidence to support previous research findings that cognitive performance of ε4-carriers is reduced compared to that of non-ε4 carriers. Further, the results indicate that the ε4 allele does not modify the beneficial effects of a university based education intervention on cognitive function over a four year period following the intervention.

Introduction

Variation in the gene responsible for the expression of the amino-acid glycoprotein Apolipoprotein E (Scott, Knott, Shaw, & Brook, 1995) is thought to contribute to variation in adult cognitive function. The allelic ε4 variant of the *APOE* gene (*APOE* ε4) has been shown to confer increased risk for Alzheimer's disease (AD), with carriers of at least one *APOE* ε4 allele more likely to develop the disease (Farrer et al., 1997; Mondadori et al., 2007). In addition, the *APOE* ε2 allele is associated with a decreased risk of AD (Rubinzstein & Easton, 1999). Inheritance of *APOE* ε4 is associated with increased risk of the formation of the pathological markers of AD, amyloid plaque deposits and neurofibrillary tangles (Bennett et al., 2005; Bennett et al., 2003a; Mondadori et al., 2007). The *APOE* ε4 allele is also more reported to be more common in cases of Mild Cognitive Impairment (Collie & Maruff, 2002). Given that

The *APOE* ε4 allele may also influence cognition in the aging population. In a review conducted by Anstey and Christensen (2000) the majority of studies were that the ε4 allele was associated with an increased rate of decline in memory processing speed, but not fluid or crystallised intelligence. Carriers of the ε4 allele have demonstrate poorer performance compared to non-carriers across a range of cognitive functions, including episodic memory (Deary et al., 2004; Flory, Manuck, Ferrell, Ryan, & Muldoon, 2000; Knight et al., 2014; Wisdom et al., 2011; Zehnder et al., 2009), working memory (Rosen, Bergeson, Putnam, Harwell, & Sutherland, 2002), visual attention (Greenwood, Sunderland, Friz, & Parasuraman, 2000), executive function (Knight et al., 2014; Wisdom et al., 2011) and general cognition (Wisdom et al., 2011). According to Wisdom et al. (2011), this decline in cognitive function in ε4-carriers becomes more pronounced with age. However, in a recent study it was found that when individuals known to develop dementia by follow-up cognitive

assessment were removed from statistical analysis, there were no detectable differences in cognitive performance between ε4-carriers and ε4-non carriers (Knight et al., 2014). Such a finding suggests that studies exploring the relationship between *APOE* allelic variation and cognitive performance may be contaminated by older adults in early stages of dementia, indicating that the ε4 allele itself may not directly contribute to variation in cognitive performance. Consistent with this, other studies report that cognitive performance does not vary based on ε4 carrier status (Donix et al., 2012; Jorm et al., 2007). However, in a later follow-up study to that of Jorm et al. (2007), an association between ε4 genotype and performance on the Mini-Mental State Examination was found, but only when controlling for risk factors such as head injury and education (Christensen et al., 2008).

However, few studies exist examining the relationship between the *APOE* ε4 allele and the rate of cognitive change over time. Knight et al. (2014) reported no significant differences in rate of decline between *APOE* ε4 present and ε4 absent individuals aged over 65 over a 10 year period. Two longitudinal studies that initially found a significant difference in rate of cognitive decline between ε4 present and ε4 absent individuals, reported that the results could in part be due to higher proportions of prodromal dementia in the ε4 present group (Praetorius, Thorvaldsson, Hassing, & Johansson, 2013; Salmon et al., 2013). In a 12-year study of older adults, Van Gerven, Van Boxtel, Ausems, Bekers, and Jolles (2012) reported no differences in rate of decline in the ε4 present and ε4 absent groups across a range of cognitive functions. However, there was a significant effect of ε4 in a task involving set-shifting, however, this occurred only in the oldest of their ε4 present groups (aged 71-82 years) (Van Gerven et al., 2012).

While aging and genetic factors are among the biggest risk factors associated with AD, a major research focus in the area of AD prevention has been to identify interventions that maximise cognitive function in later-life. The presumed underlying mechanism of this improvement is an increase in cognitive reserve (CR), a theoretical construct used to explain inter-individual variation in cognitive deficits arising from brain damage or disease (Stern, 2009). Cognitive training programs (Smith et al., 2009), physical activity (Ruscheweyh et al., 2011) and social activity (James et al., 2011) have shown promising results in increasing cognitive function in later-life. However, little is known regarding the potential influence of *APOE* allelic variation on an individuals' response to interventions designed to enhance cognitive function. If previous research findings are accurate, the lowered cognitive performance of *APOE* ε4 carriers relative to non-carriers could impart ε4 carriers with an increased potential to benefit from intervention. If genetic variation influences responsiveness to different forms of intervention, then knowledge of an individual's genetic makeup could results in individually tailored interventions to enhance protection against age-related cognitive decline.

We investigated the potential influence of the *APOE* ε4 allele on longitudinal cognitive function. In a sample of participants from the Tasmanian Healthy Brain Project (THBP) (Summers et al., 2013), identified as displaying improved cognitive reserve resulting from attending university (Lenehan et al., 2015, Under Review), we examined whether *APOE* ε4-carriers displayed a different response to the education intervention compared to non-ε4 carriers. Firstly, we hypothesised that cognitive performance would vary based on ε4 carrier status, with ε4-carriers performing worse than non-ε4 carriers at baseline and over a four year period in both the control and intervention groups. Secondly, it was also hypothesised that ε4-carriers would display enhanced beneficial effects of the university education intervention on

cognitive function as indicated by a significant group differences in slope between intervention group ε4-carriers and non-ε4 carriers.

Method

Participants

The Tasmanian Healthy Brain Project (THBP) (Summers et al., 2013) is a prospective longitudinal study of older adults engaging in university level education. The THBP sample has been recruited progressively from 2011-2014. Data analysed in the present paper is collected from 459 adults aged between 50 and 79 years who had participated in the THBP as of the 31st December 2014. Those in the intervention group (n = 359) had undertaken a minimum of 12 months part-time or full-time university study, with a minimum study load of two units at undergraduate or post graduate levels. The remaining 100 participants were a control reference group. These individuals did not take part in any tertiary level study. However, previous analysis of the control and intervention groups revealed two subclasses of individuals within each group (Lenehan et al., 2015, Under Review). To briefly summarise, CR theory posits that any improvement in cognitive function seen for the intervention group would be caused as a result of a positive effect of further education on CR. As such, we examined whether the intervention group displayed increased CR relative to the control group over the first 4 years of the THBP. Growth Mixture Modelling (GMM) revealed two latent classes of participants within each the control and the intervention groups, based on patterns of performance in current CR over time. In the control group, 55.7% of participants displayed improved CR, with the remaining 43.3% of participants displaying stable CR. The cognitive domain scores (see *analysis* below) over time of these two classes were compared using a

series of repeated measures analysis of variance (ANOVA). This revealed no significant differences in cognitive domain scores over time and consequently all 100 participants were retained and collapsed into a single control group for the current study. The majority of the intervention group displayed increased CR over time (92.5%), while the remainder displayed no change in CR (stable, 7.5%). A series of repeated measures ANOVAs revealed significant differences in cognitive performance between the two classes. As there were insufficient numbers in the stable CR class (n = 15) to analyse the group separately, these 15 people were excluded from the present analysis. Thus, in the present paper we examined the cognitive performance of two groups, a control group (n = 100) who did not undertake further education, and an intervention group (n = 344) who undertook university level education and have demonstrated a significant increase in CR over the first 4 years of the THBP (Lenehan et al., 2015, Under Review).

Participants who presented with a medical, neurological, or psychiatric disorder that could potentially impair cognition were precluded from entry into the THBP. The project was approved by the Human Research Ethics Committee (Tasmania) Network and further details of the study protocol have been previously published (see Summers et al., 2013).

Materials

Participants in the THBP completed a comprehensive testing battery. For detailed project protocols refer to (Summers et al., 2013). The Dementia Rating Scale, 2nd edition (DRS-2; Jurica et al., 2001), the Hospital Anxiety and Depression Scale (HADS; Snaith, 2003) and the Medical Health Status questionnaire (Summers et al., 2013) were administered to ensure participants were free from dementia and of sound psychological and physical health.

Estimates of pre-morbid intellectual capacity were obtained using the Wechsler Test of Adult Reading (WTAR; The Psychological Corporation, 2001).

Neuropsychological performance.

The neuropsychological test battery comprised 14 tests encompassing four broad cognitive domains: episodic memory, working memory, executive function and language processing. Composite scores were created for each cognitive domain by Principal Components Analysis consistent with an approach utilised in previous work by this group (Ward, Summers, Saunders, & Vickers, 2014). Briefly, the episodic memory score comprised Logical Memory test (LMI, LMII; Wechsler, 1997b), Rey Auditory Verbal Learning Test (RAVLT; Lezak et al., 2012) and Paired Associates Learning (PAL; Cambridge Cognition Limited, 2012). The working memory score comprised Digit Span (Wechsler, 1997a), Letter Number Sequencing (Wechsler, 1997a), Spatial Span (SSP; Cambridge Cognition Limited, 2012) and Spatial Working Memory (SWM; Cambridge Cognition Limited, 2012), the Executive Function score comprised Trail Making Test Trail B (TMT-B; Strauss et al., 2006), 24-item Victoria version Stroop Colour-Word Test (Stroop C; Strauss et al., 2006) and Rapid Visual Processing (RVP A'; Cambridge Cognition Limited, 2012). Finally, language processing score comprised Vocabulary (Wechsler, 1997a), Comprehension (Wechsler, 1997a) and Boston Naming Test (BNT; Kaplan et al., 1983). For each respective test, individual raw scores were standardised to z-scores against the sample mean and standard deviation at baseline assessment. Therefore, an individual's performance on each neuropsychological test over time is referenced against their performance at baseline in standard deviation units above or below the 50^{th} percentile. To create the domain composite scores, the z-scores from relevant tests were multiplied by the factor coefficients produced from the principal

components analyses (PCA). To this effect, cognitive domain composite scores represent decline or improvement over time relative to the sample mean at baseline.

Genotyping.

DNA was extracted from saliva samples using Oragene DNA Self-Collection KITS (DNA Genotek Inc., 2012). *APOE* genotype was determined using a one-step amplified refractory mutation system polymerase chain reaction (ARMS-PCR). The method described by Donohoe et al. (1999) was used to determine rs429358 and rs7412. PCR amplifications were undertaken in a 12 μl reaction volume that contained approximately 50 ng of genomic DNA. PCR amplicons were resolved on 2% agarose gel. Genotyping was repeated on samples to ensure accuracy.

Procedure

After obtaining consent, the elements of the full THBP test battery used in the present analysis were administered to each participant in the following order: WTAR, DRS-2, Medical Health Questionnaire, PAL, RAVLT, Logical Memory I, SSP, Digit Span, SWM, Letter Number Sequencing, Logical Memory II, Vocabulary, Comprehension, BNT, RVP A', STROOP C, TMT B, HADS concluding with DNA collection. An approximate 20 minute delay occurred between the administration of LMI and LMII. DNA data and IQ estimates (WTAR) were only collected once, at baseline. The full THBP test battery took approximately four hours to complete and subjects were encouraged to take short breaks as

needed to avoid fatigue (Summers et al., 2013). Participants were reassessed at one year intervals (± one month).

Analysis

Principal components analyses.

Initially, four separate PCAs were conducted to compute composite scores for each cognitive domain at baseline using SPSS, version 19. PCA was selected in order to reduce the number of variables while retaining as much of the original variance as possible (Conway & Huffcutt, 2003). Previous studies of the THBP have used similarly constructed composite scores (Ward, Summers, Saunders, & Vickers, 2014).The factorability of items in each cognitive domain was assessed with reference to a number of recognised criteria. Firstly, it was observed that all tests specific to each domain correlated at least .3 with at least one other test. Secondly, the Kaiser-Meyer-Olkin measure of sampling adequacy was above the recommended value of .60 (Hair et al., 1998) and in each case Bartlett's test of sphericity was significant. The diagonals of the anti-image correlation matrices (measures of sampling adequacy) were all above the .5 recommended minimum (Field, 2009). Based on these indicators, factor analysis was considered to be suitable with all 14 neuropsychological tests.

It was specified in the analysis that one component be extracted for each domain of cognitive function. Given the large sample size, item factor loadings of $\geq .3$ could be considered statistically significant (Hair et al., 1998). However, only factor loadings of $\geq .4$ were considered to have practical interpretability in the present study. The results of the PCA are presented in Table 12. Factor coefficients for each of the test scores were combined into a

single factor score using a regression method, yielding a z-score. The equation that resulted in episodic memory score = .356 (LM I) + .346 (LM II) + .305 (RAVLT) + .245 (PAL). The equation that resulted in working memory score = .397 (Letter Number Sequencing) + .376 (Digit Span) + .325 (SWM) - .306 (SSP). The equation that resulted in executive function score = .439 (Stroop C) + .424 (TMT B) - .460 (RVP A'). Finally, the equation that resulted in language processing score = .360 (Boston Naming Test) + .442 (Comprehension) + .477 (Vocabulary). To calculate domain composite scores for the subsequent time points (T1, T2, T3), baseline referenced z-scores for the relevant tests were imputed into these formula. Baseline referenced z-scores were calculated because they indicate whether the individual has improved or decline since their start point have been used in other published longitudinal studies of age-related cognitive decline (e.g. Zahodne et al., 2011).

Table 12: Principal component analysis results for composite cognitive domain scores.

Cognitive domain	Eigenvalue (variance explained)	Test Name	Mean	SD	Loading
Episodic memory	2.51 (62.65%)	LM I immediate recall total	48.31	8.30	.89
		LM II delayed recall total	30.15	6.41	.87
		RAVLT 1-5 recall total	53.14	8.86	.76
		PAL first trial memory score	18.35	3.35	.61
Working memory	2.01 (50.23%)	Letter number sequencing	11.67	2.39	.80
		Digit span	18.77	3.91	.76
		SSP span length	5.76	1.20	.65
		SWM between errors	25.63	18.58	-.61
Executive function	1.71 (57.03%)	RVP A’	.91	.05	-.79
		Stroop C time	25.94	7.53	.75
		TMT B time	59.02	19.67	.73
Language processing	1.81 (60.35%)	Vocabulary	56.90	5.78	.86
		Comprehension	26.15	3.41	.80
		Boston Naming Test	57.68	2.90	.65

Multiple group latent growth curve modelling.

Multiple group latent growth curve modelling (LGCM) was conducted using *Mplus 7.0* (Muthén & Muthén, 1998-2012) maximum likelihood estimation. Multiple group LCGA was conducted because it enables the direct comparison of the control and intervention group, for example whether the slope of the intervention group significantly departs from slope of the control group which is important in analysing the effect of the intervention. A more standard independent analysis of each group separately would not enable this essential comparison.

Initially, separate models for each cognitive domain containing only linear slopes were compared to corresponding models containing both linear and quadratic slopes to determine basic shape. For all cognitive domains these models initially included both fixed and random effects of the linear and quadratic factors. Within each cognitive domain model, parameters were free to be different for each of the four genotype groups.

In all models, time was paramatised with time scores that represented years since study entry and the intercept loadings of the four time points were fixed at one. In each model, the intercept term represented the mean of each respective cognitive domain score, the linear growth term represented the annual rate of change in score, and the quadratic growth term indicated the change in the rate of change (accelerating or decelerating change).
Next, to examine whether groups were significantly different in terms of the intercept and linear slope, comparisons were made between the best fitting freely estimated model described above and a series of constrained models for each group against the other three groups for each cognitive function. The first constrained model held the intercept term equal across the two groups involved in the comparison. The second constrained model held the linear slope terms equal between the two groups. The difference in chi-square between the

freely estimated model and the constrained models revealed whether the groups differed significantly in terms of intercept or slope.

Model fit

A number of statistics were considered in deciding whether a model was a good representation of the data. The likelihood-ratio chi-square is a popular statistic used to assess overall fit. In general a smaller, insignificant value at the level of .05 indicates a well-fitting model (Hooper et al., 2008). However, because chi-square is sensitive to sample size, the statistic can be prone to type II error in the case of large sample sizes (Hooper et al., 2008) and consequently a range of other fit indices were considered. The root mean squared error of approximation (RMSEA) is a measure of closeness of fit with values of < .7 indicating good fit and < .03 indicating excellent fit (Steiger, 2007). An RMSEA Value of ≥ .8 is considered a poor fit. Finally, comparative fit index (CFI) was also considered with Values of ≥ .95 indicative of good fit (Hu & Bentler, 1999).

Results

Descriptive Data

The sample consisted of 444 older adults, aged between 50 – 79 years at baseline (M = 60.25, SD = 6.75). Overall, the sample was of above average intelligence (M = 112.5, SD = 5.38), free from dementia (M = 11.92, SD = 2.13), and not clinically depressed (M = 2.51, SD = 2.88) or anxious (M = 5.30, SD = 3.09). Males were under-represented in the sample (32.2%), a feature common in longitudinal research in this field (Zahodne et al., 2011).

A breakdown of demographic information for each *APOE* group is presented in Table 13. A series of ANOVAs were conducted on demographic variables and revealed a single significant *APOE* genotype related effect in age, ($F_{(3, 440)}$= 6.37), p = <.001). Follow up comparisons revealed that the control non-ε4 carrier group were significantly older at baseline than the intervention ε4-carrier and non- ε4 carrier groups. However, as there were no significant correlations between age and neuropsychological performance across any of the four time points in any *APOE* group, the decision was made not to include these factors as covariates in further analyses. Cohen's (1988) cut off values were utilised with only correlations of a moderate (≥ .5) or large (≥ .8) magnitude considered meaningful given the large sample size. In addition, including age and baseline education in the model did not significantly improve the fit of the model, or dramatically alter the structure of the model, consequently the decision was made not to include age as a covariate in the analyses. Means and standard deviations for cognitive domain scores at each time point as a function of group are presented in Table 14.

Table 13: Sample demographic information as a function of *APOE* group.

	Control		Intervention	
	non-ε4 carrier *N* at T0 = 63	ε4-carrier *N* at T0 = 37	non-ε4 carrier *N* at T0 = 255	ε4-carrier *N* at T0 = 89
	M *(SD)*	M *(SD)*	M *(SD)*	M *(SD)*
Female *N* (%)	43 *(68.3)*	20 *(54.1)*	180 *(70.6)*	58 *(65.2)*
Baseline Age	63.52 *(6.13)*	60.73 *(6.11)*	59.50 *(6.85)*	59.87 *(6.53)*
Prior Education	13.51 *(2.87)*	13.57 *(2.24)*	14.39 *(2.67)*	13.96 *(2.71)*
DRS-2 AEMSS	11.97 *(3.09)*	11.81 *(2.61)*	11.84 *(3.22)*	12.17 *(2.92)*
WTAR (est. FSIQ)	113.02 *(2.33)*	111.59 *(2.34)*	112.45 *(2.22)*	112.88 *(2.43)*

HADS - Anxiety	5.43*(2.36)*	5.65 *(2.13)*	5.27 *(2.08)*	5.15 *(2.15)*
HADS - Depression	5.43 *(5.35)*	5.65 *(4.41)*	5.27 *(5.38)*	5.15 *(5.79)*

Table 14: Neuropsychological performance as a function of *APOE* group.

		Control						Intervention					
		Non-ε4 carrier			ε4 carrier			Non-ε4 carrier			ε4 carrier		
		N	M	*(SD)*	*N*	M	*SD*	N	*M*	*SD*	N	*M*	*(SD)*
Episodic Memory	T0 -Baseline	63	-.17	1.02	37	-.11	.98	254	.05	.98	89	.01	1.03
	T1	57	-.21	1.00	34	.15	.82	203	.10	1.03	69	.22	.856
	T2	46	.14	.90	20	.19	.88	145	.47	.95	54	.28	1.02
	T3	32	.29	1.00	14	.61	.87	74	.79	.91	28	.79	.88
Working Memory	T0 -Baseline	63	-.22	1.01	37	.02	1.05	253	-.01	1.00	89	.13	1.02
	T1	57	-.22	1.02	34	.16	.96	202	.01	1.01	69	.07	.94
	T2	47	.06	1.04	20	-.06	.86	146	.11	.98	54	.05	1.00
	T3	32	.07	1.23	14	-.22	.96	74	.15	1.06	28	.36	1.03
Executive Function	T0 -Baseline	63	-.03	.58	37	-.02	.65	254	.01	.65	88	.02	.51
	T1	57	-.01	.66	34	.09	.61	202	-.04	.66	68	.01	.65
	T2	47	-.14	.66	20	-.06	.53	145	-.15	1.23	53	.03	.62
	T3	31	-.13	.65	14	-.18	.44	74	.04	.70	27	-.03	.46
Language Processing	T0 -Baseline	63	-.11	1.03	37	-.13	1.06	255	.04	.96	89	.13	.97
	T1	57	-.04	1.12	35	-.04	.86	204	.14	.94	68	.33	.96
	T2	47	-.05	1.30	21	-.15	1.12	147	.35	.75	54	.36	1.00
	T3	32	.06	.76	14	-.09	1.18	74	.28	.89	28	.33	.83

Multiple Group LGCM

Adding a quadratic slope did not result in a significant improvement to model fit for any cognitive domain. Additionally, for all cognitive functions the variance associated with the linear growth term was negative and non-significant, indicating little variance in individual rates of change. Therefore, the subsequent models for all four domains included only linear fixed effects.

As shown in Table 15, significant linear improvement was evident in episodic memory for each of the four groups (Figure 12). In working memory all groups showed small, though non-significant, linear increases in scores overtime (Figure 13). A declining trend was evident in all groups for executive function (Figure 14), though this decline was only significant for the intervention ε2/ ε3 groups, which could be a result of the larger sample size in that group. While there was a non-significant decline in language processing performance in the two control groups, the two intervention groups improved scores over time (Figure 15). However, this linear increase was only significant in the intervention ε2/ ε3 group.

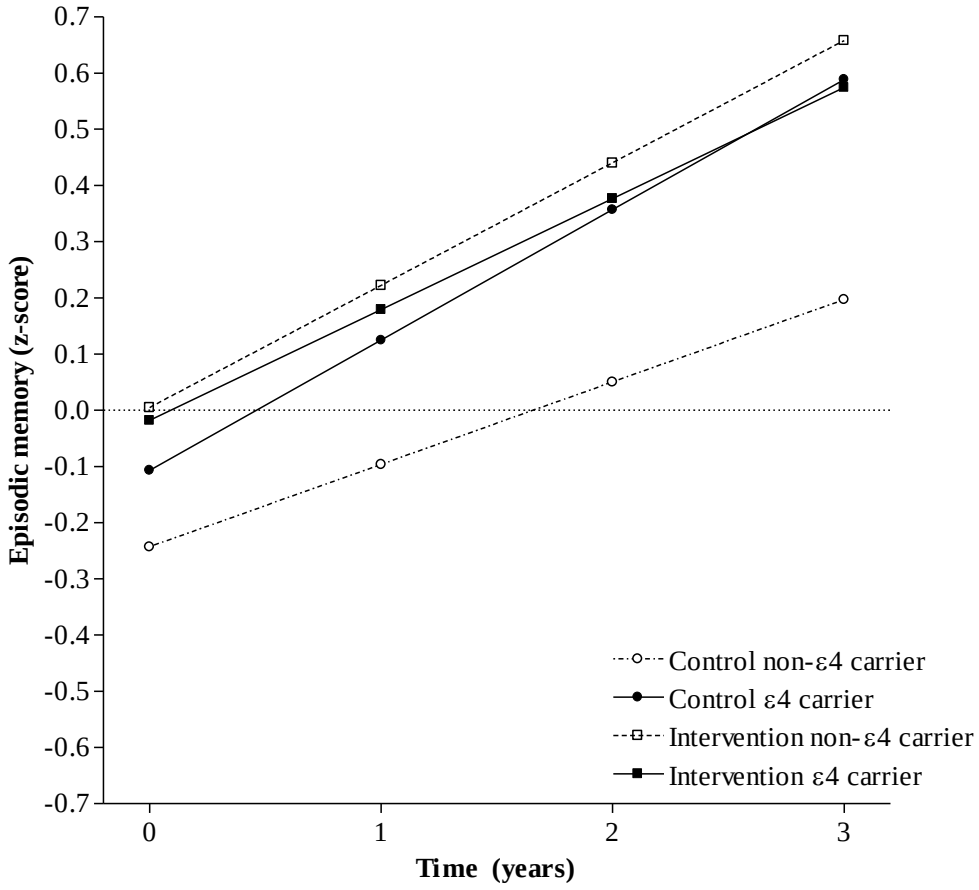

Figure 12: Model-predicted episodic memory trajectories by *APOE* group over 4 years.

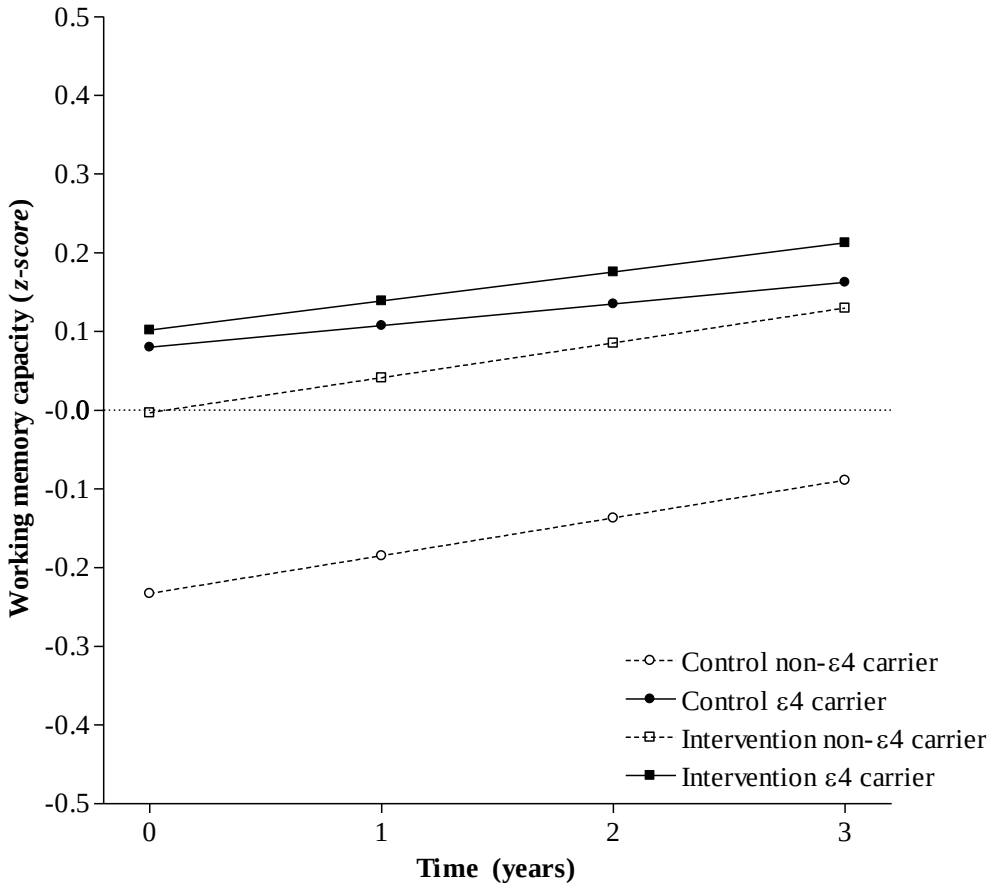

Figure 13: Model-predicted working memory trajectories by *APOE* group over 4 years.

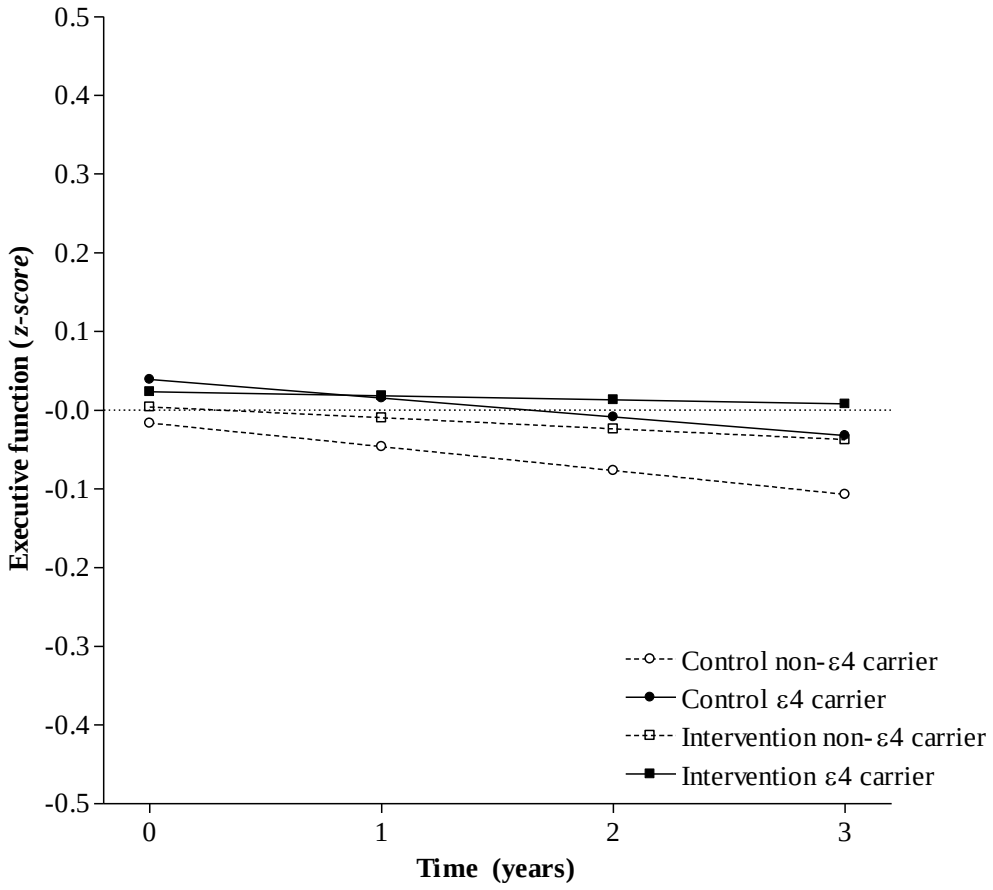

Figure 14: Model-predicted executive function trajectories by *APOE* group over 4 years.

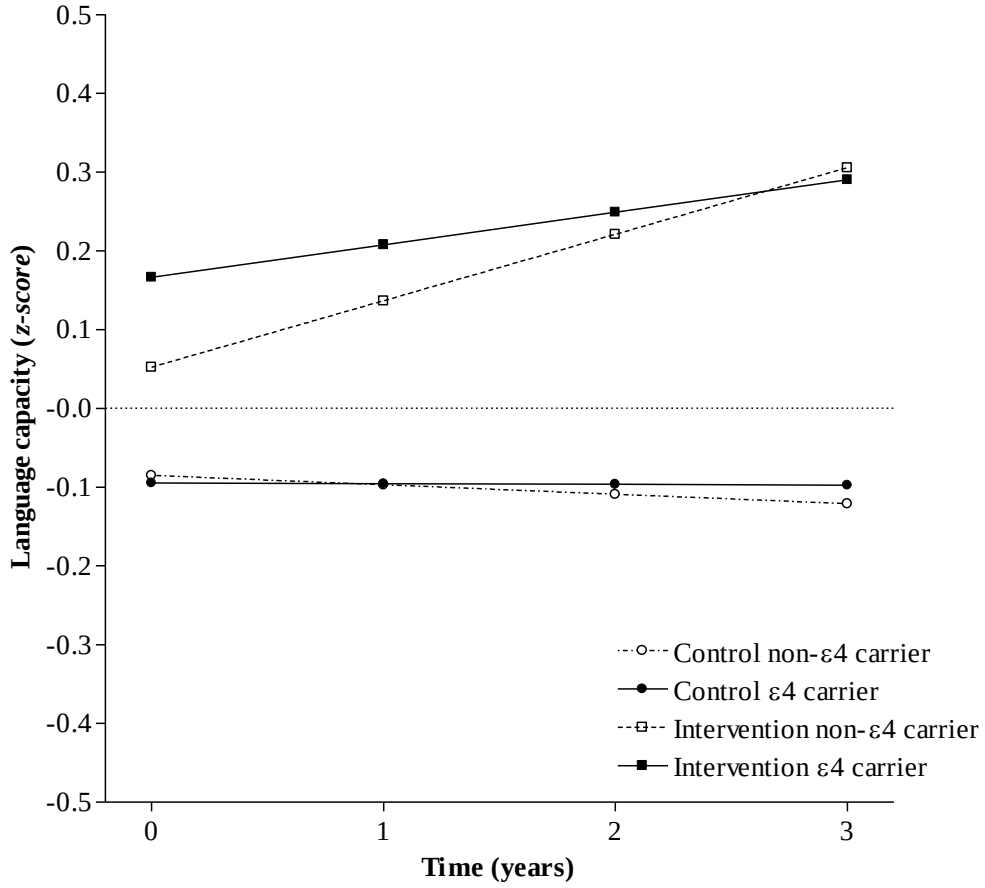

Figure 15: Model-predicted language processing trajectories by *APOE* group over 4 years.

Table 15: Estimates (SE) of group specific means for latent variables by *APOE* group.

	Control non-ε4 carrier	Control ε4-carrier	Intervention non-ε4 carrier	Intervention e4 ε4-carrier
	N = 63	*N*=37	*N* = 255	*N*=89
	Model estimates (*SE)*		Model estimates (*SE)*	
Episodic memory				
Intercept	-.243 *(.127)*	-.107 *(.143)*	.004 *(.062)*	-.018 *(.103)*
Variance	.79 *(.15)***	.61 *(.16)***	.76 *(.08)***	.80 *(.14)***
Linear growth rate	.147 *(.035)***	.232 *(.049)***	.218 *(.019)***	.197 *(.033)***
Variance	.00 *(.00)*	.00 *(.00)*	.00 *(.00)*	.00 *(.00)*
Working memory				
Intercept	-.233 *(.129)*	.080 *(.161)*	-.003 *(.062)*	.102 *(.104)*
Variance	.88 *(.17)***	.84 *(.21)***	.80 *(.08)***	.77 *(.13)***
Linear growth rate	.048 *(.032)*	.028 *(.037)*	.044 *(.018)**	.037 *(.033)*
Variance	.00 *(.00)*	.00 *(.00)*	.00 *(.00)*	.00 *(.00)*
Executive function				
Intercept	-.016 *(.069)*	.039 *(.094)*	.004 *(.039)*	.023 *(.053)*
Variance	.13 *(.04)***	.18 *(.06)***	.22 *(.03)***	.10 *(.03)***
Linear growth rate	-.030 *(.035)*	-.024 *(.037)*	-.014 *(.022)*	-.005 *(.028)*
Variance	.00 *(.00)*	.00 *(.00)*	.00 *(.00)*	.00 *(.00)*
Language processing				
Intercept	-.085 *(.130)*	-.095 *(.158)*	.052 *(.050)*	.166 *(.099)*
Variance	.93 *(.18)***	.71 *(.19)***	.54 *(.06)***	.70 *(.12)***
Linear growth rate	-.012 *(.030)*	-.001 *(.049)*	.084 *(.025)***	.041 *(.164)*
Variance	.00 *(.00)*	.00 *(.00)*	.00 *(.00)*	.00 *(.00)*

Note: $^{*}p. < .05$, $^{**}p. < .01$.

As shown in Table 16, a series of chi-square difference tests revealed only two significant group differences. Firstly, intervention ε4-carriers had a significantly higher intercept than the control non-ε4 carriers group in the working memory domain. Secondly, there was a significant difference between the slope of control group non-ε4 carriers and intervention group non-ε4 carriers in the language processing domain.

Table 16: Chi-square model comparisons of the freely estimated model with a series of constrained models to compare within and between group differences in *APOE* genotype in intercept and linear slope.

		Intercept comparisons				Linear slope comparisons			
		χ^2 freely estimated model (a)	χ^2 intercepts constrained (b)	$\Delta\chi^{2\,(df)}$ (b) - (a)	*p.*	χ^2 freely estimated model (c)	χ^2 intercepts constrained (d)	$\Delta\chi^2$ (d) – (c)	*p.*
Episodic Memory									
Control non-ε4 carrier	Control ε4 carrier	41.587	42.091	.504 (1)	>.05	41.587	43.533	1.946 (1)	>.05
	Intervention non-ε4 carrier	41.587	43.533	1.946 (1)	>.05	41.587	44.652	3.065 (1)	>.05
	Intervention ε4-carrier	41.587	43.467	1.88 (1)	>.05	41.587	42.674	1.087 (1)	>.05
Control ε4 carrier	Intervention non-ε4 carrier	41.587	42.096	.509 (1)	>.05	41.587	41.658	.071 (1)	>.05
	Intervention ε4- carrier	41.587	41.840	.253 (1)	>.05	41.587	41.921	.334 (1)	>.05
Intervention non-ε4 carrier	Intervention ε4-carrier	41.587	41.062	.065 (1)	>.05	41.587	41.864	.277 (1)	>.05
Working Memory									
Control non-ε4 carrier	Control ε4 carrier	34.35	36.61	2.26 (1)	>.05	34.35	34.521	.171 (1)	>.05
	Intervention non-ε4 carrier	34.35	36.889	2.539 (1)	>.05	34.35	34.360	.001 (1)	>.05
	Intervention ε4-carrier	34.35	38.376	4.026 (1)	<.05	34.35	34.406	.056 (1)	>.05
Control ε4 carrier	Intervention non-ε4 carrier	34.35	34.581	.231 (1)	>.05	34.35	34.513	.163 (1)	>.05
	Intervention ε4-carrier	34.35	34.363	.013 (1)	>.05	34.35	34.385	.035 (1)	>.05
Intervention non-ε4 carrier	Intervention ε4- carrier	34.35	35.109	.759 (1)	>.05	34.35	34.387	.037 (1)	>.05

Executive Function									
Control non-ε4 carrier	Control ε4-carrier	20.105	20.330	.225 (1)	>.05	20.105	20.121	.016 (1)	>.05
	Intervention non-ε4 carrier	20.105	20.172	.067 (1)	>.05	20.105	20.263	.158 (1)	>.05
	Intervention ε4-carrier	20.105	20.315	.210 (1)	>.05	20.105	20.420	.315 (1)	>.05
Control ε4 carrier	Intervention non-ε4 carrier	20.105	20.222	.117 (1)	>.05	20.105	20.159	.054 (1)	>.05
	Intervention ε4-carrier	20.105	20.126	.021 (1)	>.05	20.105	20.268	.163 (1)	>.05
Intervention non-ε4 carrier	Intervention ε4-carrier	20.105	20.192	.087 (1)	>.05	20.105	20.166	.061 (1)	>.05
Language Processing									
Control non-ε4 carrier	Control ε4-carrier	33.258	33.260	.002 (1)	>.05	33.258	33.295	.037 (1)	>.05
	Intervention non-ε4 carrier	33.258	34.192	.934 (1)	>.05	33.258	39.371	6.113 (1)	<.05
	Intervention ε4-carrier	33.258	35.614	2.356 (1)	>.05	33.258	34.843	1.585 (1)	>.05
Control ε4 carrier	Intervention non-ε4 carrier	33.258	34.015	.757 (1)	>.05	33.258	35.722	2.464 (1)	>.05
	Intervention ε4-carrier	33.258	35.193	1.935 (1)	>.05	33.258	33.809	.551 (1)	>.05
Intervention non-ε4 carrier	Intervention ε4-carrier	33.258	34.274	1.016 (1)	>.05	33.258	34.520	1.262 (1)	>.05

Discussion

The hypothesis that the cognitive performance of ε4-carriers would be lower than that of non-ε4 carriers was not supported by the results of the present study. No significant differences were found between intervention group ε4-carriers and non-ε4 carriers, or between control group ε4-carriers and non-ε4 carriers, either in baseline score or linear rate of change over time on any cognitive function. The second hypothesis, that ε4-carriers would display an enhanced benefit of education-based intervention on cognitive function, was also not supported. The linear slope of the intervention ε4-carriers was not significantly different to the slope of non-ε4 carriers across any of the four cognitive domains.

These results suggest that in a group of healthy older adults *APOE* genotype is unrelated to cognitive performance and the four year trajectory of cognitive change. This is inconsistent with previous research which reports carriers of the ε4 allele display reduced cognitive performance relative to non-ε4 carriers (Deary et al., 2004; Wisdom et al., 2011; Zehnder et al., 2009). In contrast to these previously reported results, Knight et al. (2014) recently reported that ε4 carrier status was associated with reduced cognitive function in tests of memory and executive function. However, when they repeated the same analysis with those individuals known to have developed dementia at the 10 year follow-up removed from the sample, an association between this ε4 carrier status and cognitive performance was no longer evident. This raises the possibility that in many of the studies reporting an effect of *APOE* carrier status on cognitive function, the ε4-carrier groups may have contained a sufficient proportion of individuals with prodromal stages of dementia so as to result in group differences in cognitive performance. When such individuals are excluded from an analysis

(Knight et al., 2014) then no relationship between ε4 carrier status and cognitive function remains.

This study also yielded no evidence to support the suggestion that allelic variation of the *APOE* gene moderates the capacity of an education based intervention to increase cognitive reserve in healthy older adults, at least over a four year period. However, it remains possible that the *APOE* ε4 variant may confer disadvantage over the long-term, whereby ε4-carriers may display a more rapid decline trajectory in age-related cognitive decline. If this is the case, then the lack of a finding of an ε4-associated acceleration in age-related cognitive decline may reflect the relative short time span assessed in the present study. Further, the majority of the participants in the present study are currently early-mid 60 years of age so are younger than the age at which age-related cognitive decline acceleration is reported to occur (Hedden & Gabrieli, 2004). Additionally, many cognitive functions show minimal decline over a 5-10 year period (Hedden & Gabrieli, 2004). As such the four year period of the current study may be of insufficient duration to reveal whether ε4-carriers display faster cognitive decline and to detect potential subtle interactions between *APOE* allelic variation and response to an education-based intervention.

The most prevalent limitations of this study are the small sample sizes in some groups and related to this, the variability inherent in the data. Noticeably, in the two control groups, sample sizes fall below the 100 typically preferred for latent growth modelling (Curran et al., 2010). Although models have been successfully estimated for samples sizes as small as n = 22 (e.g. Hettenlocher, Haight, Bryk, Seltzer, & Lyons, 1991), the total number of person-by-time observations influences statistical power (Curran et al., 2010). Due to the progressive recruitment of participants into the THBP over a 3 year period, the models estimated are

based on extrapolation from an incomplete dataset, where some individuals have only one or two observations over time. This may result in increased within group variability, as indicated by a larger standard error of the mean which is evident in both control groups and therefore less power to detect significant intercept and slopes. Future research will re-examine the findings of the present analysis as the complete THBP participant pool completes assessment over all time points.

In conclusion, the present study sought to examine firstly whether *APOE* allelic variation influence the rate of age-related cognitive change over time and whether *APOE* allelic variation modified an individual's responsiveness to an education intervention, shown to enhance cognitive reserve, in terms of cognitive performance. The results of the study negate previous research findings that *APOE* ε4-carriers have lower cognitive performance relative to non-ε4 carriers. Additionally, the results indicate that the ε4 allele does not modify the beneficial effects of a university based education intervention on cognitive function over the first 4 years following the intervention in healthy older adults.

Chapter 7

Does *BDNF* Val66Met polymorphism modify responsiveness to a tertiary education intervention designed to enhance cognitive reserve: The Tasmanian Healthy Brain Project

Abstract

Background: The strong link between education and cognitive performance suggests that a period of education in later-life could enhance cognitive function. However, little is known regarding whether the brain-derived neurotrophic factor (*BDNF)* Val66Met polymorphism, which has been implicated in experience-dependent plasticity and higher cognitive functions, modifies an individual's responsiveness to an education intervention.

Met*hods*: The annual cognitive performance of 444 healthy older adults, aged 50-70 years (*M* = 60.25, *SD* = 6.75) enrolled in the Tasmanian Healthy Brain Project was examined over a four year period. Episodic memory, working memory, executive function and language processing was assessed alongside *BDNF* polymorphism status (Val homozygotes and Met carriers). *BDNF* gene data was used to conduct within and between group comparisons of cognitive performance and the linear rate of cognitive change on a previously identified group of participants who undertook university study and demonstrated increased cognitive reserve (CR; intervention) and a group who did not engage in further education and did not display a stable change in CR (control).

Results: Multiple group Latent Growth Curve Modelling revealed no significant differences between intervention group *BDNF* Val homozygotes and Met carriers, or between control group Val homozygotes and Met carriers, either in baseline score or linear rate of change over time on any cognitive function. Additionally, the linear slope of the Val homozygotes was not significantly different to the slope of Met carriers across any of the four cognitive domains in the intervention group.

Conclusions: We found no evidence to support previous research findings that cognitive performance of *BDNF* Met carriers is reduced compared to that of Val homozygotes. Further, the results indicate that the Met homozygote does not modify the beneficial effects of a

university based education intervention on cognitive function over a four year period following the intervention.

Introduction

Brain derived neurotrophic factor (*BDNF*) is a gene thought to account for variance in adult cognitive function. *BDNF* is a protein which is widely distributed in the human brain, particularly in the hippocampus, cerebral cortex and the amygdala (Murer et al., 2001). *BDNF* modulates the growth of new neurons, the survival of existing neurons, as well as regulating synaptic function and plasticity (Poo, 2001). Research examining a polymorphism of the *BDNF* gene, *BDNF* Val66Met, has shown that secretion of *BDNF* in neurons is impaired when the Val66 sequence is replaced with a Met sequence (Egan et al., 2003). Consequently, carriage of the Met variant polymorphism has been linked to decreased *BDNF* availability in the brain (Egan et al., 2003).

BDNF Met carriers consistently display reduced hippocampal function (Egan et al., 2003) and volume (Pezawas et al., 2004) relative to their Val/Val homozygote counterparts. Studies of adults with preclinical AD and amnestic Mild Cognitive Impairment report that the *BDNF* Met allele is associated with greater impairment in memory and executive function relative to Val homozygotes (Lim, Villemagne, Ellis, et al., 2014; Nagata et al., 2012). Conversely, Ventriglia et al. (2002) report that homozygous Val carriers are at increased risk of developing AD. Another study found no association between *BDNF* polymorphisms and AD diagnoses (Lee et al., 2005).

Research reports inconsistency in the relationship between *BDNF* genotypes and cognitive function in healthy older adults. Some studies implicate Val66Met in decreased cognitive function of healthy adults (Dincheva, Glatt, & Lee, 2012; Li et al., 2010), with Met carriers displaying lower performance in episodic memory (Egan et al., 2003), working memory (Li et al., 2010; Richter-Schmidinger et al., 2011) and processing speed (Miyajima et al., 2008)

relative to Val/Val homozygotes. Conversely, other studies report no association between *BDNF* Met carrier status and cognitive function in healthy older adults (Persson et al., 2013; Stuart et al., 2014).

A review by Payton (2009) emphasised that to investigate the effect genetic variation on age-related cognitive decline, large scale, longitudinal studies with multiple follow-up assessments spanning decades are required. However, studies of this nature are limited. As detailed above, many studies have indicated the effect of BDNF variation on the level of performance on certain cognitive functions, but not its effect of the rate of cognitive decline over time. Erickson et al. (2008) examined the effect of BDNF variation on the change in performance on a measure of executive function in a sample of older adults, over a 10 year period. The results showed that BDNF Met carriers performed lower at baseline, but did not decline over time. On the other hand, BDNF Val carriers performed higher at baseline, but their performance declined significantly over time. Ghisletta et al. (2014) examined the effect of BDNF variation on processing speed, over a period of 13 years in a sample of elderly individuals. The results showed that BDNF Met carriers declined at a significantly faster rate, relative to BDNF Val carriers.

A few studies have examined the effect of BDNF variation, in combination with other genes or factors, on age-related cognitive decline. Lim, Villemagne, Laws, et al. (2014) investigated the effect of BDNF and APOE variation on the cognitive function of individuals classified as either having accumulated β-amyloid or not, which the authors go on to define as preclinical AD. The results showed that those with accumulated β-amyloid carrying the APOE ε4 allele and BDNF Met in combination declined significantly faster in measures of episodic memory and language, but not executive function or attention, relative to those with accumulated β-

amyloid carrying BDNF Val and APOE ε4 absent. However, in the healthy older adults included in the study (those without accumulated β-amyloid) little decline occurred over time and there were no significant differences between groups in the rate of change of performance in any cognitive function (Lim, Villemagne, Laws, et al., 2014). This suggests that the combination of APOE ε4 and BDNF Met is associated with accelerated cognitive decline in those with preclinical AD, but not healthy individuals. Another study suggests that while interactions between BDNF and catechol-O-methyltransferase (COMT) variations explain differences in level of performance on a measure of processing speed, it does not account for differences in the rate of age-related cognitive decline over time (Das et al., 2014).

While aging and genetic factors are among the biggest risk factors associated with AD, a major research focus in the area of AD prevention has been to identify approaches to maximise cognitive function in later-life. The presumed underlying mechanism of this improvement is an increase in cognitive reserve (CR), a theoretical construct used to explain inter-individual variation in cognitive deficits arising from brain damage or disease (Stern, 2009). Cognitive training programs (Smith et al., 2009), physical activity (Ruscheweyh et al., 2011) and social activity (James et al., 2011) have shown promising results in increasing cognitive function in later-life. However, we have not identified any study that examines the influence of *BDNF* genetic variation on an individuals' response to interventions designed to enhance cognitive function. If previous research findings are accurate, the lowered cognitive performance of *BDNF* Met carriers relative to Val carriers could impart Met carriers with an increased potential to benefit from intervention. If genetic variation influences responsiveness to different forms of intervention, then knowledge of an individual's genetic makeup could

results in individually tailored interventions to enhance protection against age-related cognitive decline.

We investigated the potential influence of *BDNF* Met on longitudinal cognitive function in a sample of participants participating in a prospective study of the effects of late-life education on cognitive function, the Tasmanian Healthy Brain Project (THBP) (Summers et al., 2013). We have previously reported a measureable increase in cognitive reserve evident in older adults attending university in late life relative to a healthy control group (Lenehan et al., 2015, Under Review). In the present study, we examine the influence of *BDNF* polymorphisms (Met carriers compared to Val/Val homozygotes) on response to the education intervention over four longitudinally examined cognitive domains: episodic memory, working memory, executive function and language processing. Firstly, we hypothesised that cognitive performance varies according to *BDNF* polymorphism with Met carriers performing worse than Val homozygotes at baseline and over a four year period, in both the control and intervention groups. Secondly, we hypothesised that *BDNF* Met carriers will display enhanced beneficial effects of the university education intervention on cognitive function as indicated by a significant group differences in slope between Val homozygotes and Met carriers in the intervention group.

Method

Participants

The Tasmanian Healthy Brain Project (THBP) (Summers et al., 2013) is a prospective longitudinal study of older adults engaging in university level education. The THBP sample

has been recruited progressively from 2011-2014. Data analysed in the present paper is collected from 459 adults aged between 50 and 79 years who had participated in the THBP as of the 31st December 2014. Those in the intervention group (*n* = 359) had undertaken a minimum of 12 months part-time or full-time university study, with a minimum study load of two units at undergraduate or post graduate levels. The remaining 100 participants were a control reference group. These individuals did not take part in any tertiary level study. However, previous analysis of the control and intervention groups revealed two subclasses of individuals within each group (Lenehan et al., 2015, Under Review). To briefly summarise, CR theory posits that any improvement in cognitive function seen for the intervention group would be caused as a result of a positive effect of further education on CR. As such, we examined whether the intervention group displayed increased CR relative to the control group over the first 4 years of the THBP. Growth Mixture Modelling (GMM) revealed two latent classes of participants within each the control and the intervention groups, based on patterns of performance in current CR over time. In the control group, 55.7% of participants displayed improved CR, with the remaining 43.3% of participants displaying stable CR. The cognitive domain scores (see *analysis* below) over time of these two classes were compared using a series of repeated measures analysis of variance (ANOVA). This revealed no significant differences in cognitive domain scores over time and consequently all 100 participants were retained and collapsed into a single control group for the current study. The majority of the intervention group displayed increased CR over time (92.5%), while the remainder displayed no change in CR (stable, 7.5%). A series of repeated measures ANOVAs revealed significant differences in cognitive performance between the two classes. As there were insufficient numbers in the stable CR class (*n* = 15) to analyse the group separately, these 15 people were excluded from the present analysis. Thus, in the present paper we examined the cognitive performance of two groups, a control group (*n* = 100) who did not undertake further

education, and an intervention group (n = 344) who undertook university level education and have demonstrated a significant increase in CR over the first 4 years of the THBP (Lenehan et al., 2015, Under Review).

Participants who presented with a medical, neurological, or psychiatric disorder that could potentially impair cognition were precluded from entry into the THBP. The project was approved by the Human Research Ethics Committee (Tasmania) Network and further details of the study protocol have been previously published (see Summers et al., 2013).

Materials

Participants in the THBP completed a comprehensive testing battery. For a detailed project protocol refer to Summers et al. (2013). The Dementia Rating Scale, 2nd edition (DRS-2; Jurica et al., 2001), the Hospital Anxiety and Depression Scale (HADS; Snaith, 2003) and the Medical Health Status questionnaire (Summers et al., 2013) were administered to ensure participants were free from dementia and of sound psychological and physical health. Estimates of pre-morbid intellectual capacity were obtained using the Wechsler Test of Adult Reading (WTAR; The Psychological Corporation, 2001).

Neuropsychological performance.

The neuropsychological test battery comprised 14 tests encompassing four broad cognitive domains: episodic memory, working memory, executive function and language processing. Composite scores were created for each cognitive domain by Principal Components Analysis

consistent with an approach utilised in previous work by this group (Ward, Summers, Saunders, & Vickers, 2014). Briefly, the episodic memory score comprised Logical Memory test (LMI, LMII; Wechsler, 1997b), Rey Auditory Verbal Learning Test (RAVLT; Lezak et al., 2012) and Paired Associates Learning (PAL; Cambridge Cognition Limited, 2012). The working memory score comprised Digit Span (Wechsler, 1997a), Letter Number Sequencing (Wechsler, 1997a), Spatial Span (SSP; Cambridge Cognition Limited, 2012) and Spatial Working Memory (SWM; Cambridge Cognition Limited, 2012), the Executive Function score comprised Trail Making Test Trail B (TMT-B; Strauss et al., 2006), 24-item Victoria version Stroop Colour-Word Test (Stroop C; Strauss et al., 2006) and Rapid Visual Processing (RVP A'; Cambridge Cognition Limited, 2012). Finally, language processing score comprised Vocabulary (Wechsler, 1997a), Comprehension (Wechsler, 1997a) and Boston Naming Test (BNT; Kaplan et al., 1983). For each respective test, individual raw scores were standardised to z-scores against the sample mean and standard deviation at baseline assessment. Therefore, an individual's performance on each neuropsychological test over time is referenced against their performance at baseline in standard deviation units above or below the 50^{th} percentile. To create the domain composite scores, the z-scores from relevant tests were multiplied by the factor coefficients produced from the principal components analyses (PCA). To this effect, cognitive domain composite scores represent decline or improvement over time relative to the sample mean at baseline.

Genotyping.

DNA was analysed consistent with an approach utilised in previous work by this group (Stuart et al., 2014; Ward, Summers, Saunders, Janssen, et al., 2014). Briefly, DNA was extracted from saliva samples using Oragene DNA Self-Collection KITS (DNA Genotek

Inc., 2012). *BDNF* genotype was determined using a one-step amplified refractory mutation system polymerase chain reaction (ARMS-PCR). The Method described by Sheikha, Hayden, Kryski, Smith, and Singha (2011) was used to determine Val66Met. PCR amplifications were undertaken in a 12 µl reaction volume that contained approximately 50 ng of genomic DNA. PCR amplicons were resolved on 2% agarose gel. Genotyping was repeated on samples to ensure accuracy.

Procedure

After obtaining consent the elements of the full THBP test battery used in the present analysis were administered to each participant in the following order: WTAR, DRS-2, Medical Health Questionnaire, PAL, RAVLT, Logical Memory I, SSP, Digit Span, SWM, Letter Number Sequencing, Logical Memory II, Vocabulary, Comprehension, BNT, RVP A', STROOP C, TMT B, HADS concluding with DNA. An approximate 20 minute delay occurred between the administration of LMI and LMII. DNA data and IQ estimates (WTAR) were only collected once, at baseline. The full THBP test battery took approximately four hours to complete and subjects were encouraged to take short breaks as needed to avoid fatigue (Summers et al., 2013). Participants were reassessed at one year intervals (± one month).

Analysis

Principal components analyses.

Initially, four separate PCAs were conducted to compute composite scores for each cognitive domain at baseline using SPSS, version 19. PCA was selected in order to reduce the number

of variables while retaining as much of the original variance as possible (Conway & Huffcutt, 2003). Previous studies of the THBP have used similarly constructed composite scores (Ward, Summers, Saunders, & Vickers, 2014).The factorability of items in each cognitive domain was assessed with reference to a number of recognised criteria. Firstly, it was observed that all tests specific to each domain correlated at least .3 with at least one other test. Secondly, the Kaiser-Meyer-Olkin measure of sampling adequacy was above the recommended Value of .60 (Hair et al., 1998) and in each case Bartlett's test of sphericity was significant. The diagonals of the anti-image correlation matrices (measures of sampling adequacy) were all above the .5 recommended minimum (Field, 2009). Based on these indicators, factor analysis was considered to be suitable with all 14 neuropsychological tests.

It was specified in the analysis that one component be extracted for each domain of cognitive function. Given the large sample size, item factor loadings of ≥ .3 could be considered statistically significant (Hair et al., 1998). However, only factor loadings of ≥ .4 were considered to have practical interpretability in the present study. The results of the PCA are presented in Table 17. Factor coefficients for each of the test scores were combined into a single factor score using a regression method, yielding a z-score. The equation that resulted in episodic memory score = .356 (LM I) + .346 (LM II) + .305 (RAVLT) + .245 (PAL). The equation that resulted in working memory score = .397 (Letter Number Sequencing) + .376 (Digit Span) + .325 (SWM) - .306 (SSP). The equation that resulted in executive function score = .439 (Stroop C) + .424 (TMT B) - .460 (RVP A'). Finally, the equation that resulted in language processing score = .360 (Boston Naming Test) + .442 (Comprehension) + .477 (Vocabulary). To calculate domain composite scores for the subsequent time points (T1, T2, T3), baseline referenced z-scores for the relevant tests were imputed into these formula.

Baseline referenced z-scores were calculated because they indicate whether the individual has improved or decline since their start point have been used in other published longitudinal studies of age-related cognitive decline (e.g. Zahodne et al., 2011).

Table 17: Principal component analysis results for composite cognitive domain scores.

Cognitive domain	EigenValue (variance explained)	Test Name	Mean	SD	Loading
Episodic memory	2.51 (62.65%)	LM I immediate recall total	48.31	8.30	.89
		LM II delayed recall total	30.15	6.41	.87
		RAVLT 1-5 recall total	53.14	8.86	.76
		PAL first trial memory score	18.35	3.35	.61
Working memory	2.01 (50.23%)	Letter number sequencing	11.67	2.39	.80
		Digit span	18.77	3.91	.76
		SSP span length	5.76	1.20	.65
		SWM between errors	25.63	18.58	-.61
Executive function	1.71 (57.03%)	RVP A’	.91	.05	-.79
		Stroop C time	25.94	7.53	.75
		TMT B time	59.02	19.67	.73
Language processing	1.81 (60.35%)	Vocabulary	56.90	5.78	.86
		Comprehension	26.15	3.41	.80
		Boston Naming Test	57.68	2.90	.65

Multiple group latent growth curve modelling.

Multiple group latent growth curve modelling (LGCM) was conducted using *Mplus 7.0* (Muthén & Muthén, 1998-2012) maximum likelihood estimation. Multiple group LCGA was conducted because it enables the direct comparison of the control and intervention group, for example whether the slope of the intervention group significantly departs from slope of the control group which is important in analysing the effect of the intervention. A more standard independent analysis of each group separately would not enable this essential comparison.

Initially, separate models for each cognitive domain containing only linear slopes were compared to corresponding models containing both linear and quadratic slopes to determine basic shape. For all cognitive domains these models initially included both fixed and random effects of the linear and quadratic factors. Within each cognitive domain model, parameters were free to be different for each of the four genotype groups.

In all models, time was paramatised with time scores that represented years since study entry and the intercept loadings of the four time points were fixed at one. In each model, the intercept term represented the mean of each respective cognitive domain score, the linear growth term represented the annual rate of change in score, and the quadratic growth term indicated the change in the rate of change (accelerating or decelerating change).
Next, to examine whether groups were significantly different in terms of the intercept and linear slope, comparisons were made between the best fitting freely estimated model described above and a series of constrained models for each group against the other three groups for each cognitive function. The first constrained model held the intercept term equal across the two groups involved in the comparison. The second constrained model held the linear slope terms equal between the two groups. The difference in chi-square between the

freely estimated model and the constrained models revealed whether the groups differed significantly in terms of intercept or slope.

Model fit

A number of statistics were considered in deciding whether a model was a good representation of the data. The likelihood-ratio chi-square is a popular statistic used to assess overall fit. In general a smaller, insignificant value at the level of .05 indicates a well-fitting model (Hooper et al., 2008). However, because chi-square is sensitive to sample size, the statistic can be prone to type II error in the case of large sample sizes (Hooper et al., 2008) and consequently a range of other fit indices were considered. The root mean squared error of approximation (RMSEA) is a measure of closeness of fit with Values of < .7 indicating good fit and < .03 indicating excellent fit (Steiger, 2007). An RMSEA Value of ≥ .8 is considered a poor fit. Finally, comparative fit index (CFI) was also considered with values of ≥ .95 indicative of good fit (Hu & Bentler, 1999).

Results

Descriptive Data

The sample comprised 444 older adults, aged between 50 – 79 years at baseline (M = 60.25, SD = 6.75). Overall, the sample was of above average intelligence (M = 112.5, SD = 5.38), free from dementia (M = 11.92, SD = 2.13), and not clinically depressed (M = 2.51, SD = 2.88) or anxious (M = 5.30, SD = 3.09). Males were under-represented in the sample (32.2%), a feature common in longitudinal research in this field (Zahodne et al., 2011). A breakdown of demographic information for each *BDNF* polymorphism group is presented in Table 18. A

series of ANOVAs were conducted on demographic variables and revealed a single significant *BDNF* polymorphism related effects for age ($F^{(3, 440)}$= 4.96), p = <.01). Follow up comparisons revealed the control VAL homozygous group was significantly older than the two intervention *BDNF* groups. However, as there were no significant correlations between age and neuropsychological performance across any of the four time points in any *BDNF* group, the decision was made not to include age as a covariate in further analyses. In addition, including age and baseline education in the model did not significantly improve the fit of the model, or dramatically alter the structure of the model, consequently the decision was made not to include age as a covariate in the analyses. Means and standard deviations for cognitive domain scores at each time point as a function of group are presented in Table 19.

Table 18: Sample demographic information as a function of *BDNF* polymorphism.

	Control		Intervention	
	Val homozygous *N* at T0 = 70	Met carrier *N* at T0 = 30	Val homozygous *N* at T0 = 252	Met carrier *N* at T0 = 91
	M *(SD)*	M *(SD)*	M *(SD)*	M *(SD)*
Female *N* (%)				
Baseline Age	62.44 *(6.28)*	62.60 *(6.25)*	59.70 *(6.83)*	59.31 *(6.08)*
Prior Education	13.40 *(2.65)*	13.83 *(2.67)*	14.17 *(2.68)*	14.57 *(2.69)*
DRS-2 AEMSS	11.97 *(2.25)*	11.77 *(2.36)*	11.92 *(2.14)*	11.96 *(1.99)*
WTAR (est. FSIQ)	112.33 *(5.64)*	112.87 *(3.32)*	112.52 *(5.34)*	112.67 *(5.91)*
HADS - Anxiety	5.71 *(2.94)*	5.03 *(2.82)*	5.24 *(3.28)*	5.25 *(2.72)*
HADS - Depression	2.80 *(2.34)*	2.87 *(2.32)*	2.43 *(2.35)*	2.38 *(2.05)*

Table 19: Neuropsychological performance as a function of *BDNF* group.

		Control						Intervention					
		Val homozygous			Met carrier			Val homozygous			Met carrier		
		N	M	*(SD)*	*N*	M	*SD*	*N*	*M*	*SD*	N	*M*	*(SD)*
Episodic Memory	T0 -Baseline	70	-.08	1.05	30	-.31	.88	252	.01	1.00	91	.12	.99
	T1	63	-.01	.97	28	-.22	.90	198	.10	.96	74	.21	1.06
	T2	46	.27	.85	20	-.11	.93	145	.36	.99	54	.58	.89
	T3	32	.51	.99	14	.12	.85	77	.79	.93	25	.80	.82
Working Memory	T0 -Baseline	70	-.05	1.07	30	-.31	.91	251	.03	.97	91	.04	1.03
	T1	63	-.01	1.04	28	-.24	.93	198	.03	.97	73	.011	1.05
	T2	47	.12	1.06	20	-.26	.73	146	.11	.99	54	.04	.989
	T3	32	.09	1.24	14	-.27	.93	77	.19	1.00	25	.27	1.19
Executive Function	T0 -Baseline	70	-.05	.59	30	.04	.64	251	-.02	.64	91	.11	.54
	T1	63	.09	.63	28	-.10	.65	197	-.05	.66	73	.03	.65
	T2	47	-.13	.61	20	-.07	.65	145	-.10	.75	53	-.10	1.74
	T3	32	-.14	.55	13	-.16	.69	76	-.04	.70	25	.22	.42
Language Processing	T0 -Baseline	70	-.09	1.12	30	-.19	.79	253	.07	.89	91	.06	1.15
	T1	64	.01	1.13	28	-.15	.73	199	.19	.98	73	.17	.86
	T2	48	-.01	1.38	20	-.25	.79	147	.29	.82	54	.53	.84
	T3	32	.01	1.02	14	.02	.53	77	.2557	.89	25	.41	.83

Multiple Group LGCM

Adding a quadratic slope did not result in a significant improvement to model fit for any cognitive domain. Additionally, for all cognitive functions the variance associated with the linear growth term was negative and non-significant, indicating little individual variance in rates of change. Therefore, the subsequent models for all four domains included only linear fixed effects.

As shown in Table 20, significant linear improvement was evident in episodic memory for each of the four groups (Figure 16: Model-predicted episodic memory trajectories by *BDNF* group over 4 years.Figure 16). In working memory, all groups showed small linear increases in scores overtime, though this only reached significance in the intervention *BDNF* Val homozygote group (Figure 17). In executive function, a non-significant decline in scores was evident in all groups except the intervention Met group which showed a non-significant linear increase (Figure 18). While there was a non-significant decline in language processing performance in the two control groups, the two intervention groups showed significant linear improvement (Figure 19).

Table 20: Estimates (SE) of group specific means for latent variables by *BDNF* group.

	Control		**Intervention**	
	Val homozygous	**Met carrier**	**Val homozygous**	**Met carrier**
	***N* = 70**	***N*=30**	***N* = 253**	***N*=91**
	Model estimates (*SE*)		**Model estimates (*SE*)**	
Episodic memory				
Intercept	-.120 (.117)	-.334 (.166)*	-.033 (.061)	.088 (.106)
Variance	.763 (.141)**	.638 (.187)**	.741 (.076)**	.868 (.143)**
Linear growth rate	.177 (.033)**	.133 (.057)*	.218 (.020)**	.189 (.029)**
Variance	.00 (.00)	.00 (.00)	.00 (.00)	.00 (.00)
Working memory				
Intercept	-.070 (.127)	-.265 (.160)	.025 (.062)	.023 (.102)
Variance	.964 (.174)**	.613 (.177)**	.805 (.081)**	.764 (.125)**
Linear growth rate	.053 (.032)	.006 (.042)	.046 (.019)*	.029 (.031)
Variance	.00 (.00)	.00 (.00)	.00 (.00)	.00 (.00)
Executive function				
Intercept	-.018 (.065)	-.010 (.117)	-.022 (.039)	.097 (.055)
Variance	.170 (.043)**	.165 (.075)*	.192 (.027)**	.167 (.039)**
Linear growth rate	-.006 (.029)	-.030 (.057)	-.020 (.021)	.014 (.024)
Variance	.00 (.00)	.00 (.00)	.00 (.00)	.00 (.00)
Language processing				
Intercept	-.046 (.133)	-.200 (.133)	.080 (.054)	.026 (.098)
Variance	1.02 (.192)**	.405 (.121)**	.558 (.062)**	.632 (.107)**
Linear growth rate	-.028 (.035)	.053 (.032)	.066 (.020)**	.118 (.040)**
Variance	.00 (.00)	.00 (.00)	.00 (.00)	.00 (.00)

Note: * *p.* < .05, ** *p.* < .01.

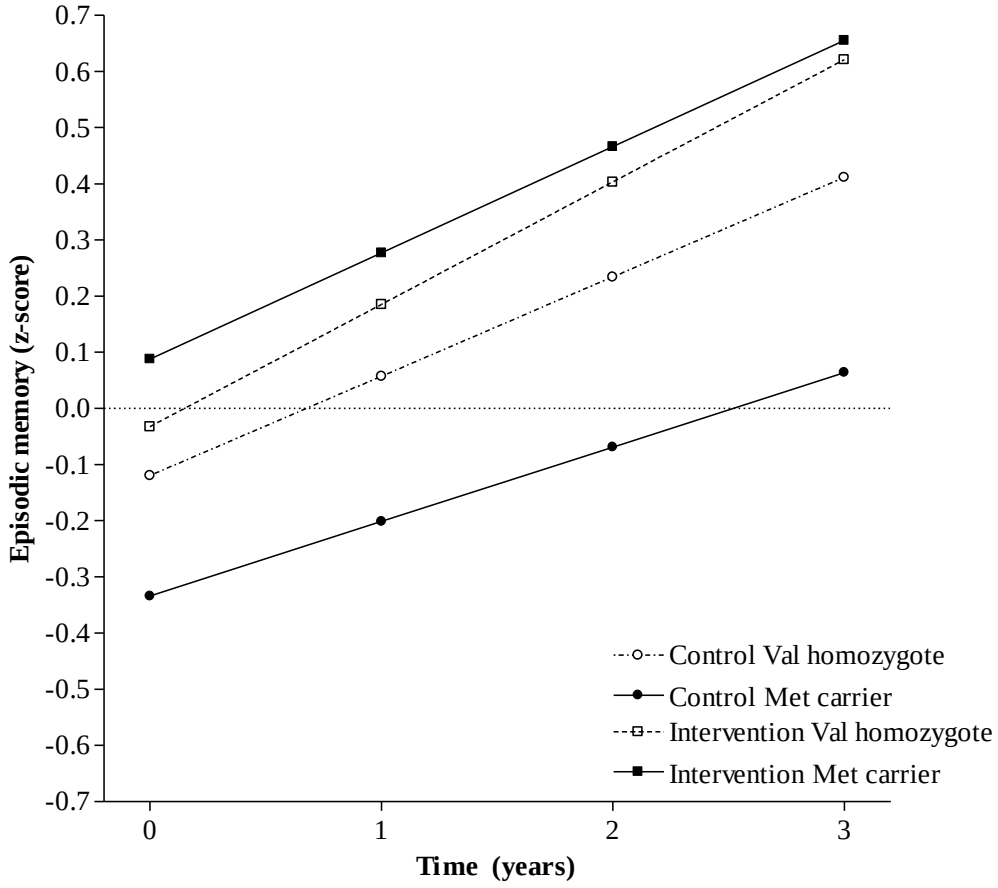

Figure 16: Model-predicted episodic memory trajectories by *BDNF* group over 4 years.

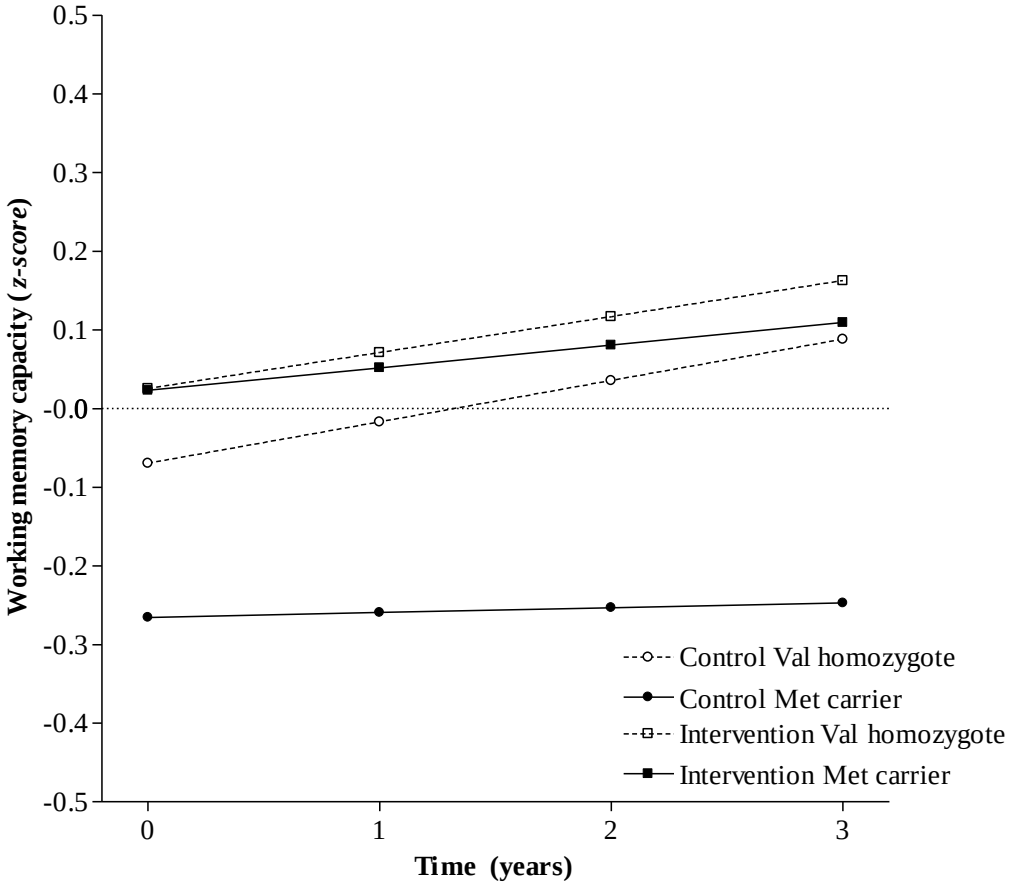

Figure 17: Model-predicted working memory trajectories by *BDNF* group over 4 years.

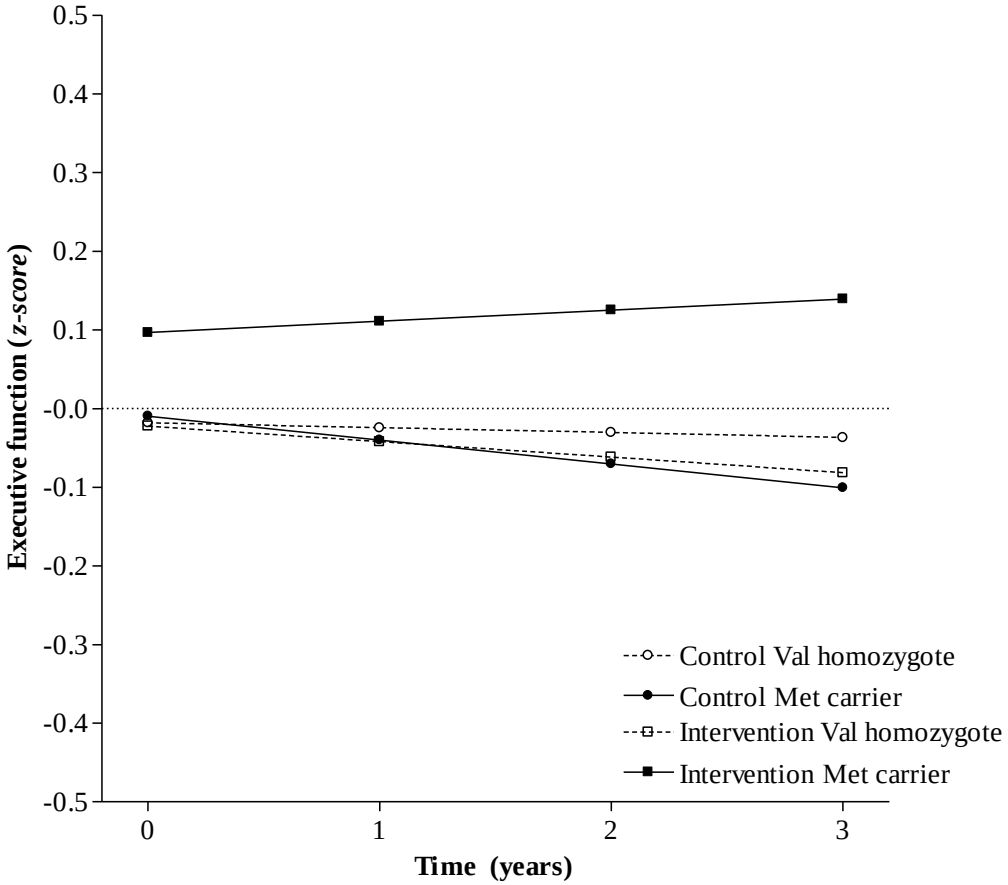

Figure 18: Model-predicted executive function trajectories by *BDNF* group over 4 years.

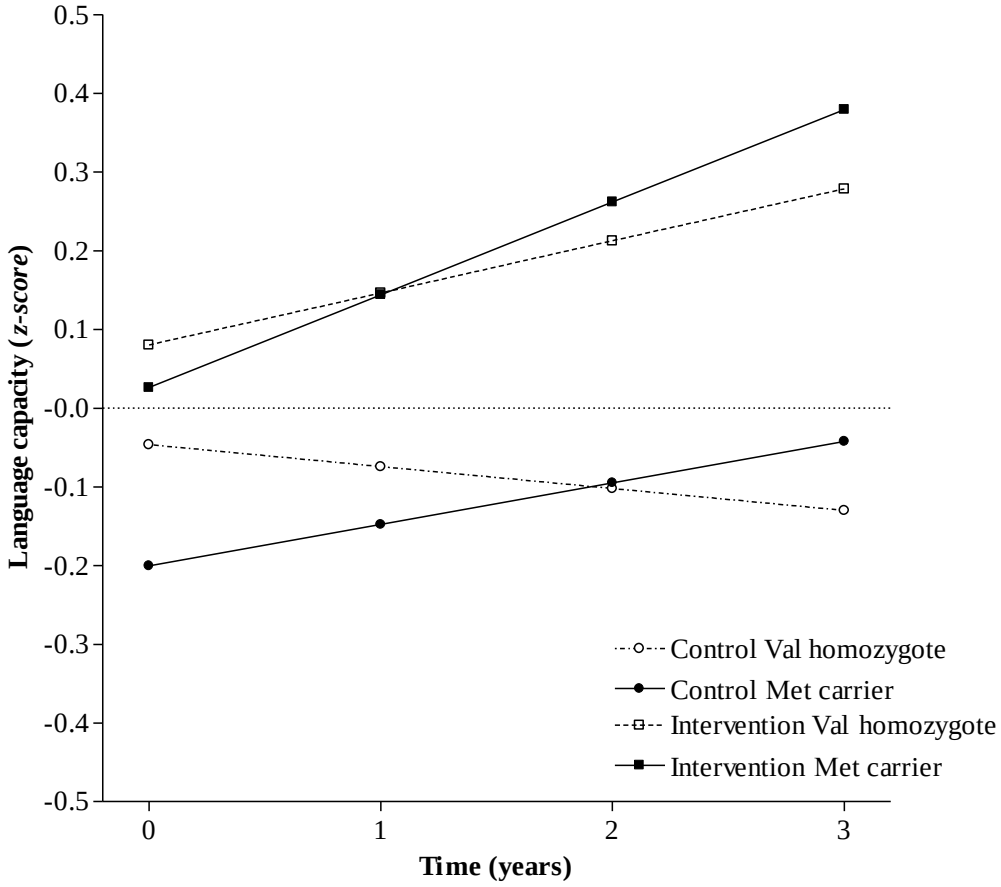

Figure 19: Model-predicted language processing trajectories by *BDNF* group over 4 years.

As shown in Table 21, the comparisons revealed only three significant group differences. The first was a significant intercept difference between the control *BDNF* Met carriers and intervention Val group, with the control Met group scoring lower at baseline compared to the intervention Val group in the episodic memory domain. The second was a significant slope difference between the control Val and the intervention Val group and the third was a significant slope difference between control Val and intervention Met groups, both in the language processing domain.

Table 21: Chi-square model comparisons of the freely estimated model with a series of constrained models to compare within and between group differences in *BDNF* genotype in intercept and linear slope.

		Intercept comparisons				**Linear slope comparisons**			
		χ^2 freely estimated model (a)	**χ^2 intercepts constrained (b)**	**$\Delta\chi^{2\,(df)}$ (b) - (a)**	***p.***	**χ^2 freely estimated model (c)**	**χ^2 intercepts constrained (d)**	**$\Delta\chi^2$ (d) – (c)**	***p.***
Episodic Memory									
Control Val	Control Met	36.077	37.179	1.102 (1)	>.05	36.077	36.541	.464 (1)	>.05
	Intervention Val	36.077	36.513	.436 (1)	>.05	36.077	37.198	1.121 (1)	>.05
	Intervention Met	36.077	37.811	1.734 (1)	>.05	36.077	36.154	.077 (1)	>.05
Control Met	Intervention Val	36.077	38.882	2.805 (1)	>.05	36.077	38.097	2.020 (1)	>.05
	Intervention Met	36.077	40.513	4.436 (1)	<.05	36.077	36.870	.793 (1)	>.05
Intervention Val	Intervention Met	36.077	37.052	.975 (1)	>.05	36.077	36.719	.642 (1)	>.05
Working Memory									
Control Val	Control Met	31.515	32.421	.906 (1)	>.05	31.515	32.283	.768 (1)	>.05
	Intervention Val	31.515	31.967	.452 (1)	>.05	31.515	31.550	.035 (1)	>.05
	Intervention Met	31.515	31.841	.326 (1)	>.05	31.515	31.807	.292 (1)	>.05
Control Met	Intervention Val	31.515	34.270	2.755 (1)	>.05	31.515	32.235	.720 (1)	>.05
	Intervention Met	31.515	33.775	2.26 (1)	>.05	31.515	31.703	.188 (1)	>.05
Intervention Val	Intervention Met	31.515	31.515	0 (1)	>.05	31.515	31.735	.220 (1)	>.05

Executive Function									
Control Val	Control Met	30.026	30.030	.004	>.05	30.026	30.168	.142	>.05
	Intervention Val	30.026	30.029	.003	>.05	30.026	30.169	.143	>.05
	Intervention Met	30.026	31.830	1.804	>.05	30.026	30.319	.293	>.05
Control Met	Intervention Val	30.026	30.037	.011	>.05	30.026	30.057	.031	>.05
	Intervention Met	30.026	30.703	.677	>.05	30.026	30.541	.515	>.05
Intervention Val	Intervention Met	30.026	33.134	3.108	>.05	30.026	31.147	1.391	>.05
Language Processing									
Control Val	Control Met	48.626	49.297	.671	>.05	48.626	51.331	2.705	>.05
	Intervention Val	48.626	49.403	.777	>.05	48.626	53.842	5.216	<.05
	Intervention Met	48.626	48.820	.194	>.05	48.626	56.076	7.45	<.01
Control Met	Intervention Val	48.626	52.298	3.672	>.05	48.626	48.749	.123	>.05
	Intervention Met	48.626	50.485	1.859	>.05	48.626	50.249	1.623	>.05
Intervention Val	Intervention Met	48.626	48.858	.232	>.05	48.626	49.960	1.334	>.05

Discussion

The hypothesis that the cognitive performance of control group *BDNF* Met carriers would be lower than that of Val homozygotes was not supported by the results of the present study. No significant differences were found between control group Val homozygotes and Met carriers, either in baseline score or linear rate of change over time on any cognitive function. The second hypothesis, that Met carriers would display an enhanced benefit of education-based intervention on cognitive function, was also not supported. The linear slope of the intervention *BDNF* Met carriers was not significantly different to the slope of Val homozygotes across any of the four cognitive domains.

These results suggest that in a group of healthy older adults *BDNF* polymorphism is unrelated to cognitive performance and the four year trajectory of cognitive change. Previous research in this area has revealed inconsistent findings, with some studies implicating *BDNF* Met in reduced cognitive function (Dincheva et al., 2012; Egan et al., 2003; Miyajima et al., 2008; Richter-Schmidinger et al., 2011) and others finding no association between *BDNF* polymorphism and cognitive performance (Persson et al., 2013; Stuart et al., 2014). The results of the current study support the notion that while *BDNF* polymorphism may influence cognitive performance in prodromal AD (Lim, Villemagne, Ellis, et al., 2014) and amnestic MCI (Nagata et al., 2012), it is not associated with cognitive function or the rate of cognitive change in healthy older adults. As the majority of the research in this area is not longitudinal in nature, it is possible that many of the studies reporting an effect of *BDNF* polymorphism on cognitive function, the Met carrier groups may have contained a sufficient proportion of individuals with prodromal stages of dementia so as to result in group differences in cognitive performance. When individuals who later developed dementia at follow-up assessment were excluded in a study examining the effect of the apolipoprotein ε4 allele on cognitive function,

the initial finding of decreased cognitive performance in ε4 carriers disappeared (Knight et al., 2014). Such findings suggest that as specific genotype groups have an increased risk of dementia, these same genotype groups may inadvertently contain an increased proportion of apparently healthy older adults who in fact are displaying prodromal dementia and thereby skew the cognitive performance of this genotype subtypes to a declining or deteriorated level relative to other subtypes.

This study also yielded no evidence to support the suggestion that variation of the *BDNF* Val66Met gene moderates the capacity of an education based intervention to increase cognitive reserve in healthy older adults, at least over a four year period. However, it remains possible that the *BDNF* Met variant may confer disadvantage over the long-term, whereby Met carriers may display a more rapid decline trajectory in age-related cognitive decline. If this is the case, then the lack of a finding of a Met-associated acceleration in age-related cognitive decline may reflect the relative short time span assessed in the present study. Further, the majority of the participants in the present study are currently early-mid 60 years of age and are therefore younger than the age at which age-related cognitive decline acceleration is reported to occur (Hedden & Gabrieli, 2004). Additionally, many cognitive functions show minimal decline over a 5-10 year period (Hedden & Gabrieli, 2004). As such, the four year period of the current study may be of insufficient duration to reveal whether Met carriers display faster cognitive decline and to detect potential subtle interactions between *BDNF* genotype and response to an education-based intervention.

The most prevalent limitations of this study are the small sample sizes in some groups and related to this, the variability inherent in the data. Noticeably, in the two control groups, sample sizes fall below the 100 typically preferred for latent growth modelling (Curran et al.,

2010). Although models have been successfully estimated for samples sizes as small as *n* = 22 (e.g. Hettenlocher et al., 1991), the total number of person-by-time observations influences statistical power (Curran et al., 2010). Due to the progressive recruitment of participants into the THBP over a 4 year period, the models estimated are based on extrapolation from an incomplete dataset, where some individuals have only one or two observations over time. This may result in increased within group variability, as indicated by a larger standard error of the mean which is evident in both control groups and therefore less power to detect significant intercept and slopes. Future research will re-examine the findings of the present analysis as the complete THBP participant pool completes assessment over all time points.

In conclusion, the present study sought to examine firstly whether *BDNF* genotype influenced the rate of age-related cognitive change overtime and secondly, whether *BDNF* genotype modified an individual's responsiveness to an education intervention, shown to enhance cognitive reserve, in terms of cognitive performance. The results of the study negate previous research findings that *BDNF* Met carriers display lower cognitive function relative to Val carriers. Additionally, the results indicate that the *BDNF* Met homozygote does not modify the beneficial effects of a university based education intervention on cognitive function over the first 4 years following the intervention in healthy older adults.

Chapter 8

APOE and *BDNF* polymorphisms do not interact to modify cognitive performance over a 4 year period: The Tasmanian Healthy Brain Project

Abstract

Background: Independently, the apolipoprotein (*APOE*) ε4 allele and the Met variant of brain-derived neurotrophic factor (*BDNF)* Val66Met have shown associations with reduced cognitive function and heightened risk of dementia in older adults. However, little is known regarding whether certain gene-gene combinations of the two confer risk or offer protection against age-related cognitive decline over time.

Methods: The annual cognitive performance of 444 healthy older adults, aged 50-70 years (*M* = 60.25, *SD* = 6.75) enrolled in the Tasmanian Healthy Brain Project was examined over a four year period. Episodic memory, working memory, executive function and language processing was assessed alongside *APOE* status (ε4 carriers and non-ε4 carriers) and *BDNF* polymorphism status (Val homozygotes and Met carriers). *APOE* and *BDNF* data was used to conduct within and between group comparisons of cognitive function and the linear rate of cognitive change on a previously identified group of healthy older adults.

Results: Multiple group Latent Growth Curve Modelling and subsequent chi-square difference tests revealed no significant differences between any of the gene-gene combinations, either in baseline score or linear rate of change over time on any cognitive function.

Conclusions: We found no evidence to suggest that specific gene-gene combinations of *APOE* and *BDNF* moderate cognitive performance or cognitive change in healthy older adults, at least over a four year period. However, it remains possible that specific gene-gene combinations may confer cognitive advantage or disadvantage over the longer term. This will become clearer as the sample ages and an acceleration of age-related cognitive decline occurs.

Introduction

Cognitive health is an important factor in maintaining quality of life and independence in older populations. Heritability studies suggest that genetic variants account to some extent, for variance in general cognitive ability throughout the lifespan (Owen et al., 2010). Two possible candidate genes for variance in cognitive function and cognitive decline in older adults, are Apolipoprotein E (*APOE*) and brain derived neurotrophic factor (*BDNF*). While both genes have been studied independently, displaying inconsistent associations with cognitive function in healthy older adults, few studies have considered whether specific gene-gene combinations confer increased risk or offer protection against age-related cognitive decline over time.

The ε4 allelic variant of the *APOE* gene (*APOE* ε4) has been associated with increased risk for Alzheimer's disease (AD), with carriers of at least one *APOE* ε4 allele up to three times more likely to develop the disease (Mondadori et al., 2007). Inheritance of *APOE* ε4 is associated with increased risk of the formation of the pathological markers of AD, amyloid plaque deposits and neurofibrillary tangles (Bennett et al., 2005; Bennett et al., 2003a; Mondadori et al., 2007). In older adults, ε4-carriers display poorer performance compared to non-ε4 carriers in episodic memory (Deary et al., 2004; Wisdom et al., 2011; Zehnder et al., 2009), executive function and general cognition (Wisdom et al., 2011). According to Wisdom et al. (2011), this decline in cognitive function in ε4-carriers becomes increasingly pronounced with age. Other studies have similarly reported no association between *APOE* ε4 carrier status and cognitive performance (Donix et al., 2012; Jorm et al., 2007). Despite its importance, few studies have investigated the effect of *APOE* ε4 carrier status on rate of cognitive decline over time.

In a 12-year study of older adults, Van Gerven et al. (2012) reported no differences in rate of decline between ε4 present and ε4 absent groups across a range of cognitive functions. However, there was a significant effect of ε4 in a task involving set-shifting, however, this occurred only in the oldest of their ε4 present groups (aged 71-82 years) (Van Gerven et al., 2012). Two longitudinal studies that initially found a significant difference in rate of cognitive decline between ε4 present and ε4 absent individuals, reported that the results could in part be due to higher proportions of prodromal dementia in the ε4 present group (Praetorius et al., 2013; Salmon et al., 2013). Similarly, a recent study reports that when individuals who had developed dementia at follow-up cognitive assessment were removed from earlier statistical analysis, there were no longer detectable differences in the rate of cognitive decline between ε4-carriers and ε4-non carriers (Knight et al., 2014). This finding raises the possibility that studies exploring the relationship between *APOE* polymorphisms and cognitive performance may be contaminated by older adults in early or prodromal stages of dementia, indicating that the ε4 allele itself may not directly contribute to variation in cognitive performance.

Similar inconsistencies exist within research regarding the association between *BDNF* and cognitive function. *BDNF* is a protein widely distributed in the human brain, particularly in the hippocampus, cerebral cortex and the amygdala (Murer et al., 2001). *BDNF* controls the growth and survival of neurons, and regulates synaptic function and plasticity (Poo, 2001). Research examining a polymorphism of the *BDNF* gene, *BDNF* Val66Met, has shown that secretion of *BDNF* in neurons is impaired when the Val66 sequence is replaced with a Met sequence (Egan et al., 2003). Consequently, the Met variant polymorphism has been linked to decreased *BDNF* availability in the brain (Egan et al., 2003).

BDNF Met carriers consistently display reduced hippocampal function (Egan et al., 2003) and volume (Pezawas et al., 2004) when compared to Val/Val homozygotes carriers. Studies of adults with preclinical AD and amnestic Mild Cognitive Impairment indicate that the *BDNF* met allele is associated with greater impairment in memory and executive function relative to Val homozygotes (Lim, Villemagne, Ellis, et al., 2014; Nagata et al., 2012). In healthy older adults, while some studies implicate the Met variant of the Val66Met in decreased performance in episodic memory (Egan et al., 2003), working memory (Richter-Schmidinger et al., 2011) and processing speed (Miyajima et al., 2008) relative to Val/Val homozygotes, other studies fail to find an association between *BDNF* Met carrier status (Persson et al., 2013; Stuart et al., 2014).

Studies that have investigated the effect of BDNF on rates of age-related cognitive decline are limited. Erickson et al. (2008) examined the effect of BDNF variation on the change in performance on a measure of executive function in a sample of older adults, over a 10 year period. The results showed that *BDNF* Met carriers performed lower at baseline, but did not decline over time. On the other hand, *BDNF* Val carriers performed higher at baseline, but their performance declined significantly over time. Ghisletta et al. (2014) examined the effect of BDNF variation on processing speed, over a period of 13 years in a sample of elderly individuals. The results showed that *BDNF* Met carriers declined at a significantly faster rate, relative to *BDNF* Val carriers.

Given the findings that variation in *APOE* and *BDNF* Met may be independently associated with increased risk for AD, lower rates of cognitive function in older age, and an increased rate of age-related cognitive decline, it is possible that these genes could interact to confer heightened risk for those who carry both the ε4 allele and *BDNF* Met. While Ward et al.

(2014) reported that episodic memory performance was highest in *APOE* ε2/*BDNF* Met allele carriers compared to all other *APOE* allele and *BDNF* polymorphism gene combinations, there was no evidence of a detrimental effect of *APOE* or *BDNF* polymorphism combinations on cognitive performance. Further, little is known regarding whether certain genetic combinations exert a beneficial or detrimental effect on cognitive functions over time. Lim, Villemagne, Laws, et al. (2014) observed that that the combination of *APOE* ε4 and *BDNF* Met is associated with accelerated cognitive decline in those with preclinical AD, but not healthy individuals. Another study suggests that while interactions between BDNF and catechol-O-methyltransferase (COMT) variations explain differences in level of performance on a measure of processing speed, it does not account for differences in the rate of age-related cognitive decline over time (Das et al., 2014).

We investigated whether certain combinations of *APOE* and *BDNF* would moderate the trajectory of cognitive performance over time, in a sample of healthy older adults. Two hypotheses were examined using four year data from the Tasmanian Healthy Brain Project (THBP; Summers et al., 2013). Firstly, we hypothesised that carriers of both risk genes (*APOE* ε4/*BDNF* Met) would display reduced cognitive function at baseline compared to other genetic combinations (*APOE* non- ε4/*BDNF* Val; *APOE* non- ε4/*BDNF* Met; & *APOE* ε4/*BDNF* Val), as evidenced by a significant baseline difference between ε4/Met carriers and the other genetic combinations. Secondly, the at-risk ε4/Met combination would display a greater decline in cognitive performance over time, as evidenced by a significant slope difference between ε4/Met carriers and the other genetic combinations.

Method

Participants

The sample comprised 444 adults aged between 50 and 79 years recruited from an existing participant pool from the THBP (Summers et al., 2013). Participants with a medical, neurological, or psychiatric history indicating a potential pre-existing impairment of cognitive performance were precluded from entry into the THBP. The project was approved by the Human Research Ethics Committee (Tasmania) Network and further details of the study protocol have been previously published (see Summers et al., 2013).

Participants were grouped according to polymorphism combinations of *APOE* (ε4 carriers or non-ε4 carriers) and *BDNF* (Val homozygotes or Met carriers) status. This resulted in four groups non-ε4/ Val carriers (*n*= 237), non-ε4/ Met carriers (*n* =81), ε4/ Val carriers (*n* =86) and ε4/ Met carriers (*n* =40).

Materials

Participants in the THBP completed a comprehensive testing battery. For a detailed project protocol refer to Summers et al. (2013). The Dementia Rating Scale, 2nd edition (DRS-2; Jurica et al., 2001), the Hospital Anxiety and Depression Scale (HADS; Snaith, 2003) and the Medical Health Status questionnaire (Summers et al., 2013) were administered to ensure participants were free from dementia and of sound psychological and physical health. Estimates of pre-morbid intellectual capacity were obtained using the Wechsler Test of Adult Reading (WTAR; The Psychological Corporation, 2001).

Neuropsychological performance.

The neuropsychological test battery comprised 14 tests encompassing four broad cognitive domains: episodic memory, working memory, executive function and language processing. Composite scores were created for each cognitive domain by Principal Components Analysis consistent with an approach utilised in previous work by this group (Ward, Summers, Saunders, & Vickers, 2014). Briefly, the episodic memory score comprised the Logical Memory test (LMI, LMII; Wechsler, 1997b), Rey Auditory Verbal Learning Test (RAVLT; Lezak et al., 2012) and Paired Associates Learning (PAL; Cambridge Cognition Limited, 2012). The working memory score comprised the Digit Span (Wechsler, 1997a), Letter Number Sequencing (Wechsler, 1997a), Spatial Span (SSP; Cambridge Cognition Limited, 2012) and Spatial Working Memory (SWM; Cambridge Cognition Limited, 2012). The Executive Function score comprised the Trail Making Test Trail B (TMT-B; Strauss et al., 2006), 24-item Victoria version Stroop Colour-Word Test (Stroop C; Strauss et al., 2006) and Rapid Visual Processing (RVP A'; Cambridge Cognition Limited, 2012). Finally, the language processing score comprised Vocabulary (Wechsler, 1997a), Comprehension (Wechsler, 1997a) and the Boston Naming Test (BNT; Kaplan et al., 1983). For each respective test, individual raw scores were standardised to z-scores against the sample mean and standard deviation at baseline assessment. Therefore, an individual's performance on each neuropsychological test over time is referenced against their performance at baseline in standard deviation units above or below the 50^{th} percentile. To create the domain composite scores, the z-scores from relevant tests were multiplied by the factor coefficients produced from the principal components analyses (PCA). To this effect, cognitive domain composite scores represent decline or improvement over time relative to the sample mean at baseline.

Genotyping.

DNA was extracted from saliva samples using Oragene DNA Self-Collection KITS (DNA Genotek DNA Genotek Inc., 2012). *APOE* genotype was determined using a one-step amplified refractory mutation system polymerase chain reaction (ARMS-PCR). The method described by Donohoe et al. (1999) was used to determine rs429358 and rs7412. *BDNF* genotype was determined using a one-step amplified refractory mutation system polymerase chain reaction (ARMS-PCR). The method described by Sheikha et al. (2011)was used to determine Val66Met. PCR amplifications were undertaken in a 12 μl reaction volume that contained approximately 50 ng of genomic DNA. PCR amplicons were resolved on 2% agarose gel. Genotyping was repeated on samples to ensure accuracy.

Procedure

After obtaining consent the test battery was administered to each participant in the following order: WTAR, DRS-2, Medical Health Questionnaire, PAL, RAVLT, Logical Memory I, SSP, Digit Span, SWM, Letter Number Sequencing, Logical Memory II, Vocabulary, Comprehension, BNT, RVP, STROOP C, TMT B, HADS and concluded with DNA collection. An approximate 20 minute delay occurred between the administration of LMI and LMII. DNA data and IQ estimates (WTAR) were only collected once, at baseline. DNA was collected using Oragnene DNA collection kits and required participants to spit 2ml of saliva into a collection vessel which was then combined with preservative fluid for subsequent analysis. Participants were instructed not to eat or drink (with the exception of water) for a period of 30 minutes prior to DNA collection. The full THBP test battery took approximately four hours to complete and subjects were encouraged to take short breaks as needed to avoid

fatigue (Summers et al., 2013). Participants were reassessed at one year intervals (± one month).

Analysis

Principal components analyses.

Initially, four separate PCAs were conducted to compute composite scores for each cognitive domain at baseline using SPSS, version 19. PCA was selected in order to reduce the number of variables while retaining as much of the original variance as possible (Conway & Huffcutt, 2003). Previous studies of the THBP have used similarly constructed composite scores (Ward, Summers, Saunders, & Vickers, 2014).The factorability of items in each cognitive domain was assessed with reference to a number of recognised criteria. Firstly, it was observed that all tests specific to each domain correlated at least .3 with at least one other test. Secondly, the Kaiser-Meyer-Olkin measure of sampling adequacy was above the recommended value of .60 (Hair et al., 1998) and in each case Bartlett's test of sphericity was significant. The diagonals of the anti-image correlation matrices (measures of sampling adequacy) were all above the .5 recommended minimum (Field, 2009). Based on these indicators, factor analysis was considered to be suitable with all 14 neuropsychological tests.

It was specified in the analysis that one component be extracted for each domain of cognitive function. Given the large sample size, item factor loadings of $\geq .3$ could be considered statistically significant (Hair et al., 1998). However, only factor loadings of $\geq .4$ were considered to have practical interpretability in the present study. The results of the PCA are presented in Table 22. Factor coefficients for each of the test scores were combined into a

single factor score using a regression method, yielding a z-score. The equation that resulted in episodic memory score = .356 (LM I) + .346 (LM II) + .305 (RAVLT) + .245 (PAL). The equation that resulted in working memory score = .397 (Letter Number Sequencing) + .376 (Digit Span) + .325 (SWM) - .306 (SSP). The equation that resulted in executive function score = .439 (Stroop C) + .424 (TMT B) - .460 (RVP A'). Finally, the equation that resulted in language processing score = .360 (Boston Naming Test) + .442 (Comprehension) + .477 (Vocabulary). To calculate domain composite scores for the subsequent time points (T1, T2, T3), baseline referenced z-scores for the relevant tests were imputed into these formula. Baseline referenced z-scores were calculated because they indicate whether the individual has improved or decline since their start point have been used in other published longitudinal studies of age-related cognitive decline (e.g. Zahodne et al., 2011).

Table 22: Principal component analysis results for composite cognitive domain scores.

Cognitive domain	Eigenvalue (variance explained)	Test Name	Mean	SD	Loading
Episodic memory	2.51 (62.65%)	LM I immediate recall total	48.31	8.30	.89
		LM II delayed recall total	30.15	6.41	.87
		RAVLT 1-5 recall total	53.14	8.86	.76
		PAL first trial memory score	18.35	3.35	.61
Working memory	2.01 (50.23%)	Letter number sequencing	11.67	2.39	.80
		Digit span	18.77	3.91	.76
		SSP span length	5.76	1.20	.65
		SWM between errors	25.63	18.58	-.614
Executive function	1.71 (57.03%)	RVP A’	.91	.05	-.79
		Stroop C time	25.94	7.53	.75
		TMT B time	59.02	19.67	.73
Language processing	1.81 (60.35%)	Vocabulary	56.90	5.78	.86
		Comprehension	26.15	3.41	.80
		Boston Naming Test	57.68	2.90	.65

Multiple group latent growth curve modelling.

Multiple group latent growth curve modelling (LGCM) was conducted using *Mplus 7.0* (Muthén & Muthén, 1998-2012) maximum likelihood estimation. Multiple group LCGA was conducted because it enables the direct comparison of the four genotype combinations. A more standard independent analysis of each group separately would not enable this essential comparison.

Initially, separate models for each cognitive domain containing only linear slopes were compared to corresponding models containing both linear and quadratic slopes to determine basic shape. For all cognitive domains these models initially included both fixed and random effects of the linear and quadratic factors. Within each cognitive domain model, parameters were free to be different for each of the four genetic groups.

In all models, time was paramatised with time scores that represented years since study entry and the intercept loadings of the four time points were fixed at one. In each model, the intercept term represented the mean of each respective cognitive domain score, the linear growth term represented the annual rate of change in score, and the quadratic growth term indicated the change in the rate of change (accelerating or decelerating change).
Next, to examine whether genetic combination groups were significantly different in terms of the intercept and linear slope, comparisons were made between the best fitting freely estimated model described above and a series of constrained models for each group against the other three groups for each cognitive function. The first constrained model held the intercept term equal across the two groups involved in the comparison. The second constrained model held the linear slope terms equal between the two groups. The difference

in chi-square between the freely estimated model and the constrained models revealed whether the groups differed significantly in terms of intercept or slope.

Model fit

A number of statistics were considered in deciding whether a model was a good representation of the data. The likelihood-ratio chi-square is a popular statistic used to assess overall fit. In general, a smaller, insignificant value at the level of .05 indicates a well-fitting model (Hooper et al., 2008). However, because chi-square is sensitive to sample size, the statistic can be prone to type II error in the case of large sample sizes (Hooper et al., 2008) and consequently a range of other fit indices were considered. The root mean squared error of approximation (RMSEA) is a measure of closeness of fit with values of < .7 indicating good fit and < .03 indicating excellent fit (Steiger, 2007). An RMSEA value of ≥ .8 is considered a poor fit. Finally, comparative fit index (CFI) was also considered with values of ≥ .95 indicative of good fit (Hu & Bentler, 1999).

Results

Descriptive Data

The sample consisted of 444 older adults, aged between 50 – 79 years at baseline (M = 60.25, SD = 6.75). Overall, the sample was of above average intelligence (M = 112.5, SD = 5.38), free from dementia (M = 11.92, SD = 2.13), and not clinically depressed (M = 2.51, SD = 2.89) or anxious (M = 5.30, SD = 3.09). Males were under-represented in the sample (32.2%), a feature common in longitudinal research in this field (Zahodne et al., 2011).

A breakdown of demographic information for each genetic combination group is presented in Table 23. A series of ANOVAs were conducted on demographic variables and revealed no significant differences between groups in terms of age, prior education, level of depression or anxiety, IQ estimate, general cognitive function or in the proportion of males/females. Additionally, there were no significant correlations between age or education and neuropsychological performance across any of the four time points in any genetic combination group. Consequently, the decision was made not to include these factors as covariates in further analyses. Cohen's (1988) cut off values were utilised with only correlations of a moderate (≥ .5) or large (≥ .8) magnitude considered meaningful given the large sample size. In addition, including age and baseline education in the model did not significantly improve the fit of the model, or dramatically alter the structure of the model, consequently the decision was made not to include age as a covariate in the analyses. Means and standard deviations for cognitive domain scores at each time point as a function of group are presented in Table 24.

Table 23: Sample demographic information as a function of *APOE* group.

	non-ε4/Val ***N* at T0 = 237**	**non-ε4/Met** ***N* at T0 = 81**	**ε4/Val** ***N* at T0 = 86**	**ε4/Met** ***N* at T0 = 40**
	M *(SD)*	**M *(SD)***	**M *(SD)***	**M *(SD)***
Female *N* (%)	167 *(70.5)*	56 *(69.1)*	55 *(64)*	23 *(57.5)*
Baseline Age	60.22 *(6.13)*	60.53 *(6.87)*	60.50 *(6.50)*	59.30 *(6.17)*
Prior Education	14.08 *(2.74)*	14.62 *(2.68)*	13.80 *(2.53)*	13.93 *(2.72)*
DRS-2 AEMSS	11.90 *(2.17)*	11.77 *(2.01)*	12.00 *(2.12)*	12.20 *(2.21)*
WTAR (est. FSIQ)	112.47 *(5.55)*	112.83 *(4.84)*	112.50 *(4.98)*	112.56 *(6.37)*
HADS - Anxiety	5.29 *(3.28)*	5.35 *(2.91)*	5.48 *(3.02)*	4.90 *(2.35)*
HADS - Depression	2.51 *(2.34)*	2.35 *(1.96)*	2.52 *(2.40)*	2.83 *(2.42)*

Table 24: Neuropsychological performance as a function of genetic combination group.

		Genetic combination group											
		non-ε4/Val			non-ε4/Met			ε4/Val			ε4/Met		
		N	M	*(SD)*	*N*	M	*SD*	N	*M*	*SD*	N	*M*	*(SD)*
Episodic Memory	T0 -Baseline	236	-.02	1.02	81	.09	.93	86	.032	.99	40	-.14	1.08
	T1	190	.01	1.01	70	.10	1.09	71	.25	.81	32	.08	.91
	T2	144	.38	.95	47	.44	.93	47	.23	1.00	27	.30	.98
	T3	80	.68	.97	26	.53	.94	29	.78	.90	13	.62	.80
Working Memory	T0 -Baseline	235	-.015	1.00	81	-.15	1.01	86	.07	1.05	40	.15	.99
	T1	190	.00	1.01	69	-.15	1.02	71	.08	.93	32	.14	.99
	T2	146	.15	1.00	47	-.07	.94	47	.02	1.00	27	.02	.91
	T3	80	.17	1.10	26	-.02	1.14	29	.12	1.02	13	.28	1.09
Executive Function	T0 -Baseline	236	-.03	.67	81	.11	.53	85	-.01	.51	40	.07	.65
	T1	189	-.04	.67	70	-.02	.613	71	.05	.59	31	.03	.73
	T2	145	-.14	.75	47	-.18	1.85	47	-.02	.61	26	.06	.56
	T3	80	-.05	.72	25	.11	.59	28	-.14	.45	13	.04	.48
Language Processing	T0 -Baseline	237	.01	.93	81	.01	1.10	86	.09	.98	40	-.01	1.04
	T1	191	.11	1.06	70	.05	.74	72	.23	.91	31	.15	1.03
	T2	147	.26	.99	47	.23	.69	48	.08	.97	27	.45	1.17
	T3	80	.23	.87	26	.16	.82	29	.06	1.09	13	.48	.56

Multiple Group LGCM

Adding a quadratic slope did not result in a significant improvement to model fit in any of the four cognitive domains. Therefore, the subsequent models for all four domains included only linear effects. Additionally, in some cases the variance associated with the linear growth term was negative and non-significant, indicating little variance in individual rates of change. In order to avoid an inadmissible model, it was necessary to fix the slope variance to zero in certain groups of some models. This applied to the ε4/Val group in the episodic memory model, all four genetic groups in the working memory model and the ε4/Met group in both the executive function and language processing models. Therefore, the models the aforementioned groups/models contain only linear fixed effects.

As shown in Table 25, significant linear improvement was evident in episodic memory for each of the four genetic combination groups (Figure 20). In working memory all genetic groups showed linear increases in scores overtime (Figure 21), however, the linear term was only significant in the non-ε4/Val group. A declining trend was evident in all genetic groups for executive function (Figure 22), though this decline was not significant. Language processing score increased in all groups except ε4/Val carrier's over time (Figure 23). However, the linear term only reached significance in the non-ε4/Val group. The growth term for the ε4/Val group (.001) showed virtually no change in score over time. Table 26 reports a series of chi-square difference tests, which revealed no significant between group differences in baseline score or linear rate of change, in episodic memory, working memory, executive function or language processing.

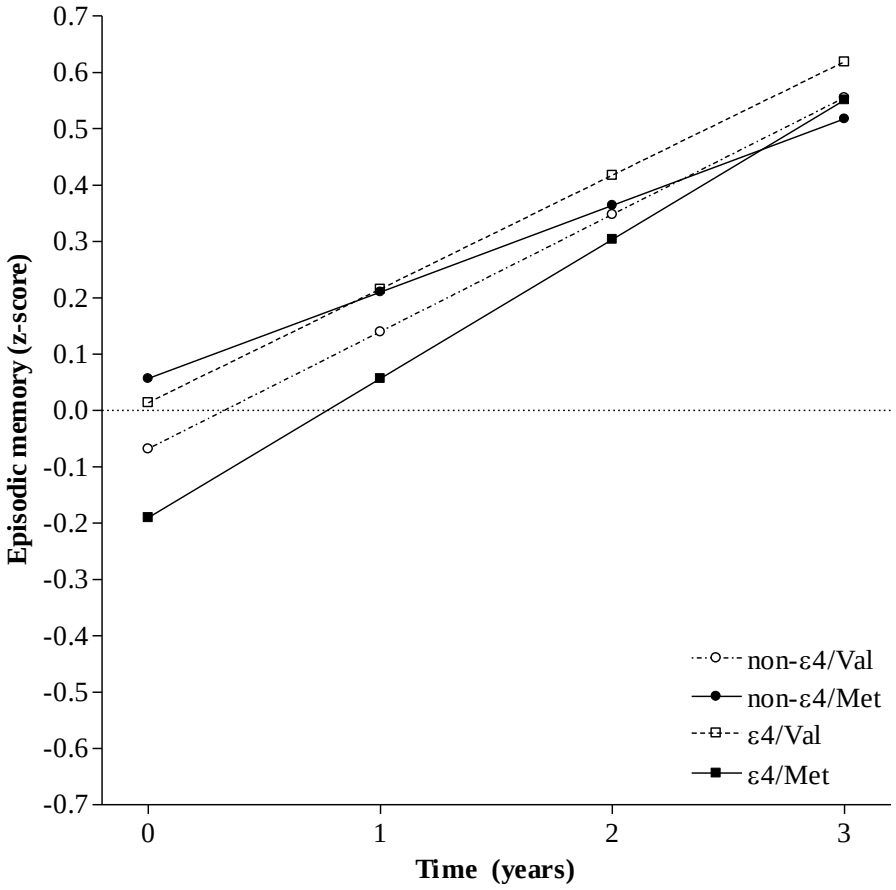

Figure 20: Model-predicted episodic memory trajectories by genetic combination group over 4 years.

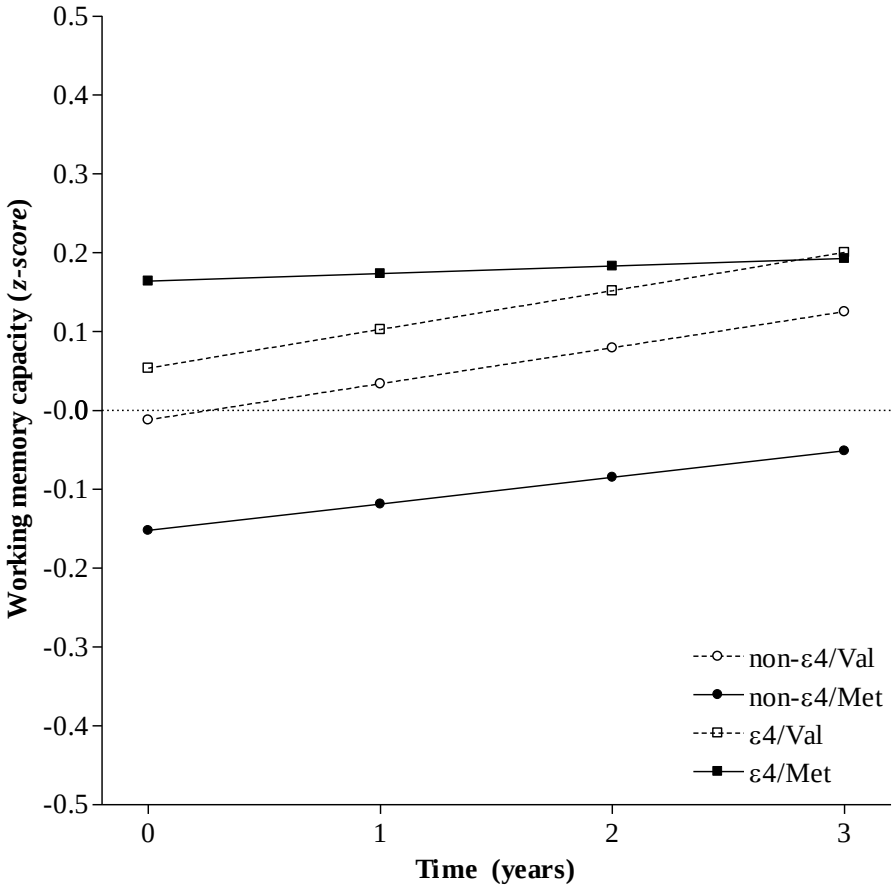

Figure 21: Model-predicted working memory trajectories by genetic combination group over 4 years.

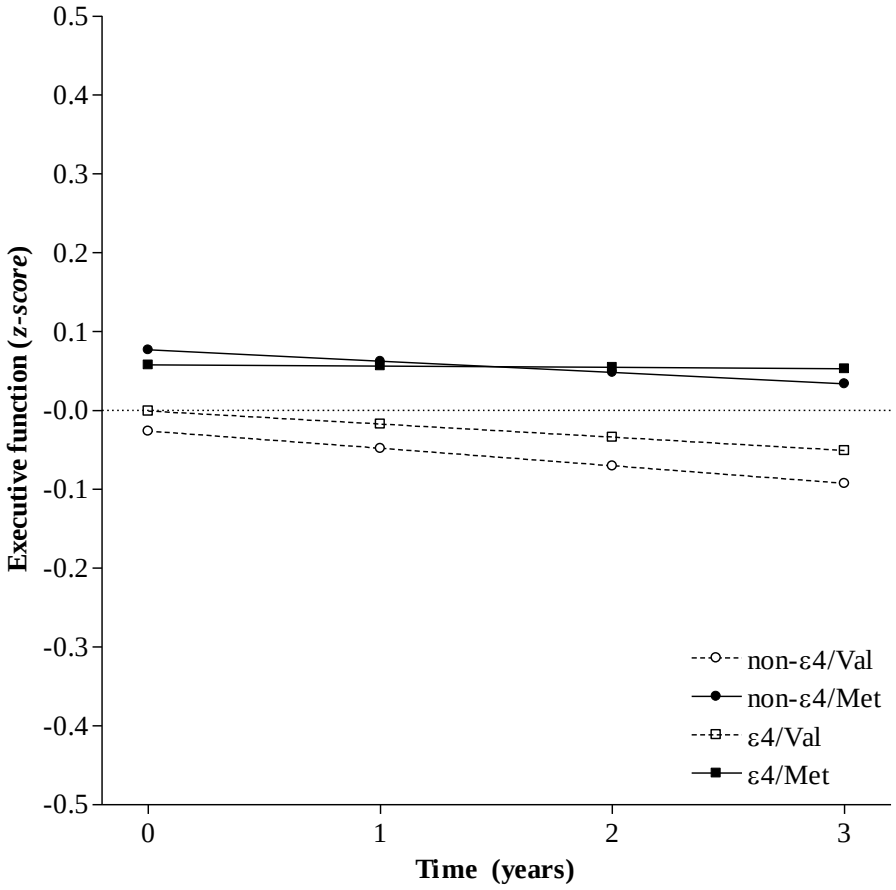

Figure 22: Model-predicted executive function trajectories by genetic combination group over 4 years.

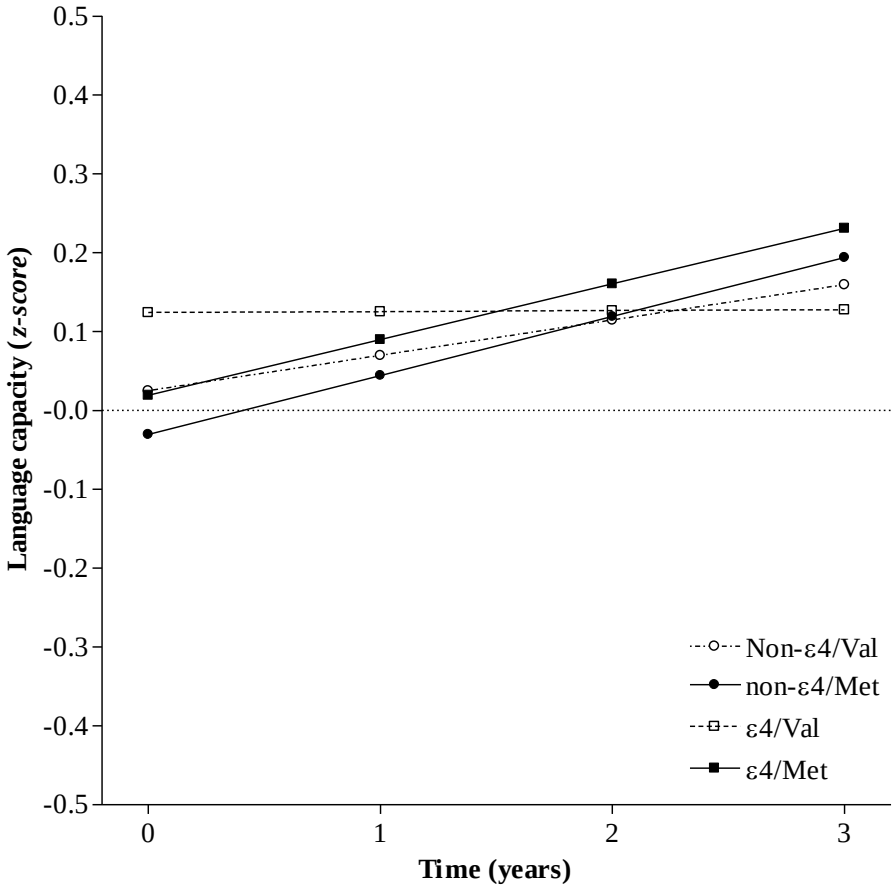

Figure 23: Model-predicted language processing trajectories by genetic combination group over 4 years.

Table 25: Estimates (SE) of group specific means for latent variables.

	Genetic combination group			
	Non-ε4/Val	**Non-ε4/Met**	**ε4/Val**	**ε4/Met**
	N = 237	**N=81**	**N = 86**	**N=40**
	Model estimates (*SE*)		**Model estimates (*SE*)**	
Episodic memory				
Intercept	-.068 *(.065)*	-.056 *(.105)*	.013 *(.099)*	-.191 *(.162)*
Variance	.786 *(.094)***	.742 *(.142)***	.722 *(.128)***	.865 *(.239)***
Linear growth rate	.208 *(.022)***	.154 *(.032)***	.202 *(.032)***	.247 *(.053)***
Variance	.020 *(.012)*	.003 *(.016)*	.000 *(.000)*	.015 *(.031)*
Working memory				
Intercept	-.012 *(.065)*	-.152 *(.106)*	.054 *(.107)*	.164 *(.149)*
Variance	.840 *(.086)***	.734 *(.127)***	.798 *(.136)***	.764 *(.187)***
Linear growth rate	.046 *(.019)**	.034 *(.030)*	.049 *(.033)*	.009 *(.044)*
Variance	.000 (.000)	000 (.000)	000 (.000)	000 (.000)
Executive function				
Intercept	-.026 *(.041)*	.077 *(.056)*	-.001 *(.055)*	.058 *(.92)*
Variance	.261 *(.048)***	.100 *(.054)*	.171 *(.054)***	.150 *(.055)***
Linear growth rate	-.022 *(.022)*	-.014 *(.038)*	-.017 *(.030)*	-.002 *(.040)*
Variance	.005 *(.020)*	.026 *(.023)*	,029 *(.014)*	.000 *(.000)*
Language processing				
Intercept	.025 *(.060)*	-.030 *(.099)*	.124 *(.100)*	.019 *(.150)*
Variance	.703 *(.093)***	.598 *(.124)***	.680 *(.144)***	.748 *(.191)***
Linear growth rate	.045 *(.023)*	.075 *(.035)**	.001*(.030)*	.071 *(.038)*
Variance	.017 *(.017)*	.013 *(.013)*	.004 *(.013)*	.000 *(.000)*

Note: $^{*}p. < .05$, $^{**}p. < .01$.

Table 26: Chi-square model comparisons of the freely estimated model with a series of constrained models to compare genetic group in intercept and linear slope.

		Intercept comparisons				Linear slope comparisons			
		χ^2 freely estimated model (a)	χ^2 intercepts constrained (b)	$\Delta\chi^{2\,(df)}$ (b) - (a)	*p*	χ^2 freely estimated model (c)	χ^2 intercepts constrained (d)	$\Delta\chi^2$ (d) – (c)	*p.*
Episodic Memory									
Non-ε4/VAL	Non-ε4/Met	47.185	48.200	1.015 (1)	>.05	47.185	49.159	1.974 (1)	>.05
	ε4/Val carrier	47.185	47.660	.475 (1)	>.05	47.185	47.209	.024 (1)	>.05
	ε4/Met	47.185	47.675	.490 (1)	>.05	47.185	47.655	.047 (1)	>.05
Non-ε4/Met	ε4/Val	47.185	47.273	.088 (1)	>.05	47.185	48.323	1.138 (1)	>.05
	ε4/Met	47.185	48.807	1.622 (1)	>.05	47.185	49.464	2.279 (1)	>.05
ε4/Val	ε4/Met	47.185	48.332	1.147 (1)	>.05	47.185	47.721	.536 (1)	>.05
Working Memory									
Non-ε4/VAL	Non-ε4/Met	32.139	33.408	.269 (1)	>.05	32.139	32.256	.117 (1)	>.05
	ε4/Val carrier	32.139	32.413	.274 (1)	>.05	32.139	32.146	.007 (1)	>.05
	ε4/Met	32.139	33.304	1.165 (1)	>.05	32.139	32.712	.573 (1)	>.05
Non-ε4/Met	ε4/Val	32.139	34.001	1.862 (1)	>.05	32.139	32.258	.119 (1)	>.05
	ε4/Met	32.139	35.097	2.958 (1)	>.05	32.139	32.348	.209 (1)	>.05
ε4/Val	ε4/Met	32.139	35.501	3.362 (1)	>.05	32.139	32.657	.518 (1)	>.05

Executive Function									
Non-ε4/VAL	Non-ε4/Met	17.925	20.130	2.205 (1)	>.05	17.925	17.957	.032 (1)	>.05
	ε4/Val carrier	17.925	18.605	.068 (1)	>.05	17.925	17.947	.022 (1)	>.05
	ε4/Met	17.925	18.613	.688 (1)	>.05	17.925	18.126	.201 (1)	>.05
Non-ε4/Met	ε4/Val	17.925	18.907	.982 (1)	>.05	17.925	17.928	.003 (1)	>.05
	ε4/Met	17.925	17.956	.031 (1)	>.05	17.925	17.978	.053 (1)	>.05
ε4/Val	ε4/Met	17.925	18.222	.297 (1)	>.05	17.925	18.015	.090 (1)	>.05
Language Processing									
Non-ε4/VAL	Non-ε4/Met	32.260	32.488	.228 (1)	>.05	32.260	32.768	.508 (1)	>.05
	ε4/Val carrier	32.260	32.979	.719 (1)	>.05	32.260	33.574	1.314 (1)	>.05
	ε4/Met	32.260	32.261	001 (1)	>.05	32.260	32.600	.340 (1)	>.05
Non-ε4/Met	ε4/Val	32.260	33.457	1.197 (1)	>.05	32.260	34.771	2.511 (1)	>.05
	ε4/Met	32.260	32.336	.076 (1)	>.05	32.260	32.266	.006 (1)	>.05
ε4/Val	ε4/Met	32.260	32.595	.335 (1)	>.05	32.260	34.349	2.089 (1)	>.05

Discussion

The hypothesis that carriers of both risk genes (ε4/ Met) would display reduced cognitive function at baseline compared to other genetic combinations (non- ε4/ Val, non- ε4/ Met, & ε4/ Val) was not supported by the results of the present study. No significant differences were found between carriers of both the ε4 allele and the Met polymorphism and any genetic combinations of *APOE* or *BDNF* across any of the four cognitive domains. The second hypothesis that the ε4/Met combination would have poorer performance over time was also not supported. Although there was a trend towards the ε4/ Met having substantially less growth compared to other groups in the working memory domain, the linear slope of the ε4/Met group was not significantly different to the slope of other genetic combinations across any of the four cognitive domains.

These results suggest that in a group of healthy older adults possessing the *APOE* ε4 allele and the *BDNF* Met gene does not amount to greater risk of cognitive decline or reduced cognitive function compared to other genetic combinations of the two candidate genes for variance in adult cognitive function. It seems that no combination of these two genes confers disadvantage or advantage, at least in terms of cognitive function over a four year period. This is somewhat consistent with previous research which also found that *APOE* and *BDNF* combinations were not associated with working memory, executive function and language processing performance (Ward, Summers, Saunders, Janssen, et al., 2014). However, the authors did report that of all genetic combinations, episodic memory performance was highest in *APOE* ε2/*BDNF* Met allele carriers.

Other research has mainly looked at single gene effects of either *APOE* or *BDNF* and reported largely inconsistent findings. While multiple studies report reduced cognitive

function for *APOE* ε4 carriers compared to non- ε4 carriers, (e.g. Deary et al., 2004; Wisdom et al., 2011; Zehnder et al., 2009), there are as many reporting no association between *APOE* carrier status and cognitive performance or decline in older age (e.g. Donix et al., 2012; Jorm et al., 2007; Knight et al., 2014). Research examining the relationship between *BDNF* polymorphisms and cognitive function is equally contradictory, some studies reporting poorer cognitive performance for Met carriers compared to Val carriers (e.g. Egan et al., 2003; Miyajima et al., 2008; Richter-Schmidinger et al., 2011) and others not (Persson et al., 2013; Stuart et al., 2014).

More generally speaking, longitudinal evidence from twin studies lends limited support to the heritability of cognitive function in the elderly. For example, a twin study by Lee et al. (2010) suggests that genetic influences seem stable over time on the level of performance for the younger old (mean age 65 years) (Lee et al., 2010). However, as people age (70s and 80s) the influence of environmental factors on cognitive performance increases and the relative influence of genetic factors decreases (Lee et al., 2010). Additionally, evidence suggests that as people age, environmental factors become more influential and genetic influences become less of a contributing factor to rates of age-related cognitive decline (Lee et al., 2010). Such findings highlight the importance of mentally stimulating activities and enriched environments as people age in order to ensure that cognitive function is enhanced.

Another potential factor which could account for the discrepant findings of research in this area is the possible inclusion of people with the prodromal stages of dementia.
Knight et al. (2014) recently reported that ε4 carrier status was associated with reduced cognitive function in tests of memory and executive function. However, when they repeated the same analysis with those individuals known to have developed dementia at the 10 year

follow-up removed from the sample, the association between ε4 carrier status and cognitive performance was no longer evident. This raises the possibility that in many of the studies reporting an effect of *APOE* carrier status or *BDNF* carrier status on cognitive function, the ε4-carrier and Met-carrier groups may have contained a sufficient proportion of individuals with prodromal stages of dementia so as to result in group differences in cognitive performance. As the majority of the research in this field is cross-sectional in design, it is possible that the samples could have contained different proportions of early-stage or prodromal dementia. If such individuals were excluded from an analysis, such as in the case of the research by Knight et al. (2014), there may no longer be a relationship between either the ε4 carrier status or Met polymorphism and cognitive function.

This study contains two major limitations. Firstly, the majority of the participants in the present study are currently early-mid 60 years of age and are therefore younger than the age at which age-related cognitive decline acceleration is reported to occur (Hedden & Gabrieli, 2004). Additionally, many cognitive functions show minimal decline over a 5-10 year period (Hedden & Gabrieli, 2004). As such the four year period of the current study may be of insufficient duration to reveal whether certain combination of *APOE* and *BDNF* are beneficial or detrimental over the long term. The second limitation is the small sample size in the ε4/Met group, which fell below the 100 typically preferred for latent growth modelling (Curran et al., 2010). Although models have been successfully estimated for samples sizes as small as $n = 22$ (e.g Hettenlocher et al., 1991), the total number of person-by-time observations influences statistical power (Curran et al., 2010). Due to the progressive recruitment of participants into the THBP over a 3 year period, the models estimated are based on extrapolation from an incomplete dataset, where some individuals have only one or two observations over time. This may result in increased within group variability, as

indicated by a larger standard error of the mean which is evident in the ε4/Met group and therefore less power to detect significant intercept and slopes. Future research will re-examine the findings of the present analysis as the complete THBP participant pool completes assessment over all time points.

In conclusion, the present study sought to examine whether certain genetic combinations of *APOE* and *BDNF* offer any beneficial or detrimental effects for cognitive function in healthy older adults. The results indicated no evidence to suggest that certain combinations of *APOE* and *BDNF* moderate cognitive performance or cognitive change, at least over a four year period. A longer follow-up duration will reveal the more long term influences of genetic combinations, particularly as the mean age of the sample increases and accelerated decline begins to occur across cognitive functions.

Chapter 9

General Discussion

The Tasmanian Healthy Brain Project (THBP) is a world first prospective study examining whether university level education in later-life delays age-related cognitive decline. The aim of the current thesis was to assess the potential benefits of a tertiary based education intervention on cognitive reserve and cognitive function in a group of healthy older adults over a four year period. The potential influence of *APOE* and *BDNF* genetic subtypes was also examined to determine whether these genes modified the beneficial effects of the intervention.

Chapter 4

Background

One non-pharmacological approach to reducing the risk of rapid age-related cognitive decline and Alzheimer's disease is to increase CR. CR describes how individual differences in life experience modify the functional impact of brain pathology (Stern, 2009). Literature suggests that education is a key contributor to CR (Bennett et al., 2003b; Dufouil et al., 2003; Valenzuela & Sachdev 2006), and that higher levels of education in early adulthood is associated with superior cognitive performance in later life (Anstey & Christensen, 2000; Lenehan et al., 2015), presumably due to having higher levels of CR. Despite this, the potential benefit of an education based intervention has not been examined by prior research. Chapter 4 represents an investigation of a tertiary based intervention designed to enhance CR in healthy older adults. This was explored by measuring dynamic change in CR after accounting for pre-existing CR using two recently published factor analysis defined measures, prior CR and current CR (Ward, Summers, Saunders, & Vickers, 2014). Evidence that CR was enhanced in a greater proportion of the intervention group, relative to any

increase in CR in a control group who did not engage in further education, would suggest that a period of tertiary education can be used as an intervention to enhance CR in later-life.

Key Findings

Findings revealed that within the intervention group 92.5% of the sample displayed a significant increase in current CR over the four year period. In the control group, the proportion who displayed enhanced CR was less (55.7%). The increase in CR seen in this subgroup of control participants was evident in those individuals who displayed below average CR at baseline. This increase may reflect unreported involvement in mentally stimulating activities outside of the THBP protocol. However, it is not uncommon for a sizeable proportion of a control group to display improvement on cognitive outcomes (e.g. Ball et al., 2002) in other intervention based research. Perhaps by nature, involvement in a study investigating the potential benefits of tertiary study in later-life may motivate all participants to be more mentally engaged in general. The findings presented in Chapter 4 are consistent with other research reporting cognitive benefits from other mentally stimulating activities, such as cognitive training programs (Ball et al., 2002) and physical interventions (Kramer et al., 1999), presumably through the positive effect these activities have on building CR.

Overall the findings of Chapter 4 indicate that that the overwhelming majority of healthy older adults who engaged in some degree of university level education for at least 12 months displayed a measureable and significant increase in proxy measures of CR over the 4 year study period.

Chapter 5

Background

Early life educational attainment shows a consistent association with cognitive performance in later-life (Anstey & Christensen, 2000; Lenehan et al., 2015). A small number of studies also suggest that higher levels of education are associated with a reduced rate of cognitive decline over time (Alley et al., 2007; Bosma et al., 2003; Cullum et al., 2000). This presumably occurs due to underlying higher levels of CR. While we have demonstrated that further education leads to a measurable and significant increase in current CR in the intervention group (Chapter 4), chapter 5 represents an examination of whether this increase in CR corresponds to changes in neuropsychological test performance. This was explored by comparing the performance of the control group and the intervention group over time across four domains of cognitive function: episodic memory, working memory, executive function and language processing. Evidence of superior performance in the intervention group or a reduced rate of change over time (greater improvement or less decline over time) would suggest that the increase in CR resulted in increased neuropsychological function.

Key Findings

The first key finding of Chapter 5 was that there was no significant decline in any of the four cognitive domains over time in either group. As such, the focus shifted to identifying whether there was significant improvement in the intervention group relative to controls. The second key finding was that language processing capacity was the only cognitive domain in which significant differences were detected between the control group and intervention group. The intervention group performing higher at baseline compared to controls and in addition while

the intervention group displayed a significant linear increase in language processing capacity, the control group displayed no significant change in performance over the four year period. This is a significant finding given that lower levels of language function is associated with both greater decline in cognitive function (Farias et al., 2012) and cognitive impairment (Riley et al., 2005) in later life.

While a number of studies have shown an associated between higher educational attainment and cognitive performance in later life (Der et al., 2010; Van Dijk et al., 2008; Zahodne et al., 2011), the same studies fail to find an effect of education on the rate of age-related cognitive decline. Though such studies are not directly comparable to the present study, they demonstrate that even if the rate of change does not differ substantially between those who received an intervention and those that did not, the improvement in CR previously identified in the THBP cohort (Lenehan et al., 2015, Under Review) may be sufficient to reduce the rate of ARCD over the medium to longer term and may exert a level of protection of cognitive function in the presence of neurodegeneration.

Overall these findings suggest that in a group of older adults who display a significant increase in CR subsequent to attending university, there is a commensurate increase in language processing capacity not observed in a control reference group.

Chapter 6

Background

One of the candidate genes for adult variance in cognitive function is *APOE*. Carriers of the ε4 allele have demonstrated poorer performance compared to non-carriers across a range of cognitive functions (e.g. Deary et al., 2004; Zehnder et al., 2009). Few studies exist examining the relationship between the *APOE* ε4 allele and the rate of cognitive change over time. Of the few studies that have, most report that the ε4 is associated with accerlated age-related cognitive function (Knight et al., 2014; Praetorius et al., 2013; Van Gerven et al., 2012). However, all studies acknowledge that the reported that the results could in part be due to higher proportions of prodromal dementia in the ε4 present group.

However, little is known regarding the potential influence of *APOE* allelic variation on an individuals' response to interventions designed to enhance cognitive function. If previous research findings are accurate, the lowered cognitive performance of *APOE* ε4 carriers relative to non-carriers could impart ε4 carriers with an increased potential to benefit from intervention. Chapter 6 aimed to examine whether *APOE* ε4-carriers displayed a different response to the education intervention compared to non-ε4 carriers. This was explored through further dividing the control and intervention groups based on *APOE* allele (ε4 carriers and non-ε4 carriers) and comparing cognitive domain baseline scores and linear growth rates of each group over time. A significant group difference in cognitive domain scores over time in the intervention group would indicate that *APOE* ε4 carrier status played an important role in responsiveness to the education based intervention.

Key Findings

The findings revealed no significant differences in baseline scores on any cognitive function between control group ε4-carriers and non-ε4 carriers, or between intervention group ε4-carriers and non-ε4 carriers. Contrary to previous research findings (Deary et al., 2004; Zehnder et al., 2009), this indicates no evidence to suggest that the cognitive performance of ε4-carriers is reduced compared to that of non-ε4 carriers. Additionally, the linear slope of the intervention ε4-carriers was not significantly different to the slope of the intervention non-ε4 carriers across any of the four cognitive domains. This finding indicates that the ε4 allele does not modify the beneficial effects of a university based education intervention on cognitive function over a four year period following the intervention. As many of the studies reporting an effect of *APOE* carrier status on cognitive function are cross-sectional in nature (Flory et al., 2000; Rosen et al., 2002; Wisdom et al., 2011), the ε4-carrier groups may have contained a sufficient proportion of individuals with prodromal stages of dementia so as to result in group differences in cognitive performance. A recent paper with a 10 year follow-up period showed that when such individuals were excluded from subsequent analysis the previous relationship evident between ε4 carrier status and cognitive function disappeared (Knight et al., 2014). However, it remains possible that the *APOE* ε4 variant may confer disadvantage over the long-term, whereby ε4-carriers may display a more rapid decline trajectory in age-related cognitive decline. If this is the case, then the lack of a finding of an ε4-associated acceleration in age-related cognitive decline may reflect the relative short time span assessed in the present study

Chapter 7

Background

Another of the candidate genes for variance in adult cognitive function is *BDNF*. Some studies implicate Val66Met in decreased cognitive function of healthy adults (Dincheva, Glatt, & Lee, 2012), with Met carriers displaying lower performance relative to Val/Val homozygotes across a range of cognitive functions (Egan et al., 2003; Miyajima et al., 2008; Richter-Schmidinger et al., 2011). If previous research findings are accurate, the lowered cognitive performance of *BDNF* Met carriers relative to Val carriers could impart Met carriers with an increased potential to benefit from intervention. Chapter 7 aimed to examine whether *BDNF* Met displayed a different response to the education intervention compared to Val carriers. This was explored through further dividing the control and intervention groups based on *BDNF* carrier status (Val carriers and Met carriers) and comparing cognitive domain baseline scores and linear growth rates of each group over time. A significant group difference in cognitive domain scores overtime in the intervention group would indicate that *BDNF* carrier status played an important role in responsiveness to the education based intervention.

Key Findings

The findings revealed no significant differences in baseline scores on any cognitive function between control group Val carriers and Met carriers, or between intervention group Val carriers and Met carriers. Contrary to previous research findings (Egan et al., 2003; Erickson et al., 2008; Richter-Schmidinger et al., 2011), this indicates no evidence to suggest that the cognitive performance of Met carriers is reduced compared to that of Val carriers.

Additionally, the linear slope of the intervention Met carriers was not significantly different to the slope of the intervention Val carriers across any of the four cognitive domains. There is a scarcity of previous research examining a potential association between BDNF variation and change in cognitive performance over time. However, the current result is inconsistent with the results of Ghisletta et al. (2014) who found that BDNF Met carriers declined at a faster rate relative to Val carriers on a measure of processing speed.

The present finding indicates that the Met polymorphism does not modify the beneficial effects of a university based education intervention on cognitive function over a four year period following the intervention. However, it remains possible that the *BDNF* Met variant may confer disadvantage over the long-term, whereby Met carriers may display a more rapid decline trajectory in age-related cognitive decline. If this is the case, then the lack of a finding of a Met-associated acceleration in age-related cognitive decline may reflect the relative short time span assessed in the present study and the fact that little cognitive decline occurred overall in the sample.

Chapter 8

Background

Apolipoprotein E (*APOE*) and brain derived neurotrophic factor (*BDNF*) are candidate genes for variance in cognitive function and cognitive decline in older adults. Previous research findings are inconsistent regarding whether in isolation these genes are associated with cognitive performance and cognitive change over time (Anstey & Christensen, 2000; Payton, 2009). Chapter 6 and Chapter 7 showed no difference in responsiveness to the education

intervention based on *APOE* or *BDNF*. However, it remains possible that specific gene-gene combinations confer increased risk or offer protection against age-related cognitive decline over time. Ward et al. (2014) reported no evidence of a detrimental effect of *APOE* or *BDNF* polymorphism combinations on cognitive performance. Lim, Villemagne, Laws, et al. (2014) observed that that the combination of *APOE* ε4 and *BDNF* Met is associated with accelerated cognitive decline in those with preclinical AD, but not healthy individuals. However, there is a scarcity of research in this area. .

Chapter 8 aimed to examine whether certain combinations of *APOE* and *BDNF* would moderate the trajectory of cognitive performance over time, in a sample of healthy older adults. This was explored through dividing the entire sample into four genetic groups (*APOE* non- ε4/*BDNF* Val; *APOE* non- ε4/*BDNF* Met; *APOE* ε4/*BDNF* Val, & *APOE* ε4/*BDNF* Met). All groups were compared in terms of baseline scores and linear rate of change in scores over time across four cognitive functions (episodic memory, working memory, executive function and language processing). Significantly lower baseline scores and a reduced rate of change in scores over time in the *APOE* ε4/*BDNF* Met group differences would indicate that carriers of both risk genes are at a relative disadvantage compared to other genetic combinations.

Key Findings

No significant baseline differences, or differences in the rate of change over time, were found between carriers of both the ε4 allele and the Met polymorphism and any genetic combinations of *APOE* or *BDNF* across any of the four cognitive domains. This is somewhat consistent with previous research by (Ward, Summers, Saunders, Janssen, et al., 2014)who

found no evidence of a detrimental effect of *APOE* or *BDNF* polymorphism combinations on cognitive performance. Although there was a trend towards the ε4/ Met having substantially less growth compared to other groups in the working memory domain, the linear slope of the ε4/Met group was not significantly different to the slope of other genetic combinations across any of the four cognitive domains. This finding is consistent with the research of Lim, Villemagne, Laws, et al. (2014), who observed that that the combination of *APOE* ε4 and *BDNF* Met is associated with accelerated cognitive decline in those with preclinical AD, but not healthy individuals.

These results suggest that in a group of healthy older adults possessing the *APOE* ε4 allele and the *BDNF* Met gene does not amount to greater risk of cognitive decline or reduced cognitive function compared to other genetic combinations of the two candidate genes for variance in adult cognitive function. Some evidence suggests that as people age, environmental factors become more influential and genetic influences become less of a contributing factor to rates of age-related cognitive decline (Lee et al., 2010), which could account for the results of the present study. In addition, it is possible that the longitudinal studies which have previously reported associations between genes and cognitive function could have unknowingly contained higher proportions of prodromal dementia (Knight et al., 2014; Praetorius et al., 2013; Salmon et al., 2013).

The results of the current study indicate that no combination of *APOE* /*BDNF* subtypes confer disadvantage or advantage, at least in terms of cognitive function over a four year period. However, it is possible that this result could change when the sample ages sufficiently and we see accelerate age-related cognitive decline set in.

Implications

Tertiary Education is a Viable Way to Enhance CR in Later-Life

Identifying interventions that enhance cognitive function in later-life is of great importance in order to delay or prevent the onset of Alzheimer's disease and reduce the rate of rapid age-related cognitive decline. The findings of the present thesis imply that later-life tertiary education is a viable way to maximise cognitive reserve. This is an important finding given that higher levels of CR are associated with superior cognitive performance (e.g. Anstey & Christensen, 2000) and a reduction in dementia risk (Valenzuela & Sachdev 2006). This is especially pertinent considering that projections estimate that Australia will be facing approximately 1.7 million dementia cases by mid-century (Access Economics, 2009). A period of tertiary education in later-life, of at least two undergraduate units over consecutive semesters, enhanced CR in 92.5% of the sample who received the intervention. Further, of the small number of participants in the intervention group who displayed no change in CR over time while attending university, CR was already higher than average. This finding provides tentative evidence that individuals with already high levels of current CR may lack the capacity for further increases in current CR. This indicates that people could potentially be screened for suitability to an intervention program, based on their level of prior CR. Interventions could then target those people most likely to reap CR benefit from additional complex mental stimulation.

Potential mechanisms underlying CR change.

A number of recent research findings support the idea that it may be possible to enhance CR in older adults, and consequently slow age-related cognitive decline and delay the onset of age related diseases such as AD (Stern, 2013). Stern (2013) a pioneer in the field, has recommended that in order to achieve this, future research should study the effect of multiple intervention strategies such as cognitive, physical and social based interventions over a long period of time (Stern, 2013). As an intervention, a period of tertiary study is not only intellectual in nature but also social, and consequently is quite unique and complex. The increase in cognitive reserve found by this study is likely underpinned by an increase in the neural networks underlying the wide variety of cognitive functions involved in engaging in formal education, such as executive functions (attention, flexibility, impulse control, and perceptual speed), memory and language skills. The complex and mentally stimulating environment provided by tertiary study may have contributed to enhancing the efficiency and capacity of neural networks, as well as equipped individuals with greater flexibility in the neural networks enlisted to perform particular tasks (Stern, 2013).

The Validity of Prior CR and Current CR

The results of the present thesis provide evidence for the construct validity of the multidimensional proxy measures of prior and current CR developed by the THBP team (Ward, Summers, Saunders, & Vickers, 2014). The finding that a significantly greater proportion of the education intervention displayed increased CR than observed in the control group lends support that the multidimensional measure of current CR is assessing cognitive reserve.

Education in Later-Life has Measurable Cognitive Benefits

Another key implication of findings of this thesis is that a period of education results in measureable improvement in language processing capacity. The language processing measure of the THBP, which comprised vocabulary and other acquired knowledge based tasks, can be considered to tap into crystallised knowledge. No such benefit was found for the fluid abilities reflected in the episodic memory or working memory domains of cognitive function. This suggests that the increase in CR observed in older adults undertaking the university education intervention resulted in an increase in crystallised knowledge but not fluid abilities. Given that lower levels of linguistic capacity in late life is associated with a greater rate of decline in cognitive function (Farias et al., 2012), later-life cognitive impairments (Riley et al., 2005) and the presence of the hallmark characteristics of Alzheimer's dementia (Snowdon et al., 1996); the observed increase in language processing capacity over time may offer important protection against the ageing process. In conjunction with the finding of enhanced CR in older adults undertaking university study, the enhancement of language processing capacity may reduce the risk of dementia in the individual or reduce the functional impact of dementia in the presence of neuropathology.

Life Experiences More Important than Genetics

The two genetic variants reported in research to be related to reduced cognitive function, *APOE* ε4 and *BDNF* Met, were found to not be associated with reduced cognitive function or rate of change over time either independently or in combination (e.g. carrying both *APOE* ε4

and *BDNF* Met) in the present study. This implies that environmental factors might be more important than genetics in determining cognitive function in later-life. Longitudinal evidence from twin studies also lends limited support to the heritability of cognitive function in the elderly. For example, a twin study reported that genetic influences are stable over time on the level of performance for the younger old adult (mean age 65 years) (Lee et al., 2010). However, with increasing age (70s and 80s), the influence of environmental factors on cognitive performance increases and the relative influence of genetic factors decreases (Lee et al., 2010). Further evidence suggests that as people age environmental factors become more influential and genetic influences become less of a contributing factor to rates of age-related cognitive decline (Lee et al., 2010). These findings highlight the importance of mentally stimulating activities and enriched environments as people age in order to ensure that cognitive function is maximised.

Genetic Variance does not Modify Responsiveness to an Intervention

APOE and *BDNF* carrier status did not modify responsiveness to the intervention in the present sample. It was hypothesised that those carrying the *APOE* ε4 allele or the *BDNF* Met polymorphism would benefit more from the education based intervention due to the reduced cognitive abilities previously reported by research, however, the results do not support the hypothesis. Carriage of a particular *APOE* or *BDNF* subtype did not confer advantage or disadvantage in terms of cognitive scores over the four year period. This implies that despite carrying "risk" genes for cognitive variance, these individuals will potentially benefit from an education intervention as much as those individuals not carrying such risk genes. However, it is important to note that genetic variance might become of greater importance as the THBP sample ages. It cannot be ruled out by the present research that at the age-point when an

acceleration in age-related cognitive decline occurs that *APOE* and *BDNF* carrier status may contribute to an exacerbation or reduction in the rate of age-related cognitive decline. As the present research does not demonstrate evidence of cognitive decline in the sample, these results are silent on the potential impact of genotype on the rate and timing of onset of age-related cognitive decline.

Limitations

Study Duration and Age of Participants

As the sample in the present series of studies has a mean age in the early-mid 60s, the absence of significant cognitive decline across any of the four cognitive domains assessed suggests that neuropsychological performance needs to be monitored over a longer period of time. Overall, the sample is younger than the age at which age-related cognitive decline acceleration is reported to occur (Hedden & Gabrieli, 2004). Longitudinal research indicates that accelerated decline in most functions begins in a person's late 60s (Hedden & Gabrieli, 2004). Additionally, many cognitive functions show minimal decline over a 5-10 year period (Hedden & Gabrieli, 2004). As such the four year duration of the study in combination with the comparatively young age of the older adults involved is a key limitation because it has been unable to capture age-related cognitive decline. In this context, any improvement above and beyond that displayed by the control group was considered a benefit of the intervention resulting from the underlying improvement in CR. Consequently, the study cannot comment on whether the intervention led to a reduced rate of age-related cognitive decline or whether certain genes or genetic combinations modify responsiveness to the education intervention or confer greater risk or protection from age-related cognitive decline. It will be informative as

future research follows this sample of adults as they age into their late 60s and 70s in order to evaluate the full impact of the intervention in the context of significant age-related cognitive decline.

Sample Size and Extrapolation of Incomplete Data Set

For some of the analyses contained within this thesis, the sample sizes were small. In the analysis of the influence of *APOE* in chapter 6 and the analysis of the influence of *BDNF* in chapter 7 sample sizes in the control groups fell below the 100 typically preferred for latent growth modelling (Curran et al., 2010). Smaller sample sizes reduce the total number of person-by-time observations which influences statistical power (Curran et al., 2010). Due to the progressive recruitment of participants into the THBP over a 3 year period, the models estimated using growth modelling techniques are based on extrapolation from an incomplete dataset, where some individuals have only one or two observations over time. This may result in increased within group variance, as indicated by a larger standard error of the mean and therefore less power to detect significant intercept and slopes. It will be useful for future research to re-examine the findings of the present thesis, particularly those involving smaller sample sizes, when the complete THBP participant pool finishes assessment over all time points.

Self-Selection Bias

The voluntary recruitment of participants into the THBP may have unavoidably led to a more highly educated sample than exists in the wider community of same aged individuals.

Entrance into university requires at least a Year 12 education. However, to enable the broadest range of participants to be involved in the THBP, participants were able to complete a university bridging program in order to meet university entry prerequisites. Despite this, the mean number of years of education attainment was over 14, suggesting most participants had undertaken post-secondary school education prior to commencing the THBP. In contrast, the average number of years of education completed by Australian adults born in the 1950s and 1960s is approximately 11.7 – 11.9 years (Kelley & Evans, 1996). The participants involved in the THBP are also likely to place greater interest and value in further education than the wider community. However, this sample bias does not discredit the study because the THBP is designed to determine whether increased mental activity in later life is beneficial to various cognitive functions in an aging population. As such, the THBP has utilised higher education as the vehicle for complex mental activity. The finding of increased cognitive reserve and language function is evidence of an effect of increased mental activity that could be achieved through the pursuit of mentally stimulating activities other than higher level education, such as more informal community adult education programs.

Sensitivity of Neuropsychological Battery

A limitation associated with the repeat testing protocol adopted by the THBP is test sensitivity over time. Performance can vary overtime due to measurement error rather than a genuine change in neuropsychological performance. It is difficult to determine what level of change in scores represents a reliable change. In the context of normal age-related cognitive decline, the magnitude of change in neuropsychological test scores is likely to increase exponentially. Thus, in the earlier years of the THBP, while the sample is below the age at which rapid age-related cognitive decline is likely to be observed, differences in

neuropsychological assessment scores may not be of sufficient size to detect significance. By nature, neuropsychological tests are designed to detect clinically significant change, not sub-clinical changes. This is an issue in the current sample due to insufficient task difficulty for a normal, healthy older adult and as such ceiling effects can be observed. This highlights a need for more sensitive tests that are able to detect subclinical changes in healthy adult populations in order to discriminate different levels of cognitive function.

Possible Dose Dependent Effect of Education

This thesis has not examined the potential effect of dose of education or type of education on the degree of cognitive change. There are considerable challenges in developing a unit measure of education in order to determine dose. It is possible to identify existing study load (an existing university metric). In our sample a 12 month full time undergraduate load is quantified as 100%; with the typical student undertaking 8 units each of 12.5% load weighting. However, such a load weighting does not translate readily into a "unit of education dose" as it is conceivable that the mental activity required (i.e. education dose) varies across undergraduate year level and may vary between units in different disciplines at the same undergraduate year level. An additional complication then arises as to whether a 12.5% unit load at undergraduate level has the same "education dose" as a 12.5% unit load at a postgraduate level. Consequently, it is a future goal of the THBP to explore the feasibility of developing a standardised metric unit of "education dose" that accounts for this variability and enables dose dependent effects of education to be measured in our sample.

Conclusion

This thesis has demonstrated that cognitive reserve can be increased in a sample of healthy older adults through attending university for a brief period. This is an exciting result because higher levels of brain efficiency offers protection to individuals against rapid-age related cognitive decline and dementia. A secondary benefit of the intervention was that language function significantly improved in the sample. Given that lower levels of linguistic capacity in later life is associated with a greater rate of decline in cognitive function, cognitive impairments and the presence of the hallmark characteristics of Alzheimer's dementia, higher levels of language processing capacity may offer important protection against the ageing process. Finally, within the duration of the study it appears that *APOE* or *BDNF* subtypes do not modify responsiveness to the intervention.

While not everyone in the community is interested or capable of further study at the tertiary level in later-life, the findings of this thesis provide support for the beneficial effect of engaging in complex mentally stimulating activities on enhancing CR and cognitive function. For example, mentally stimulating activities could come in the form of short courses offered to older adults in the community. In the context of Australia's rapidly increasing aging population and consequent incidence of dementia, it is imperative to implement programs shown to enhance cognitive health in later-life. Studying not only exercises the mind through learning new information, it also incorporates high-level social engagement which in itself contributes to enhanced cognitive function (e.g. Fratiglioni et al., 2004; Seeman et al., 2001). A further positive finding is that individuals carrying genetic risk factors for dementia and cognitive decline (*APOE* ε4 allele and/or *BDNF* Met polymorphism) also benefit as much from engaging in complex mental activity.

It will be important to continue to study this cohort in order to examine potential differences in neuropsychological scores between the control group and the intervention group in the presence of age-related cognitive decline. Only then will evidence emerge as to whether an education based intervention is sufficient to reduce the rate of age-related cognitive decline and thereby potentially delay the onset of cognitive impairment or dementia. Similarly, a longer follow-up duration will enable the impact of genetic variance on both the trajectory of age-related cognitive decline and the response to the tertiary education intervention to be examined.

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Appendix A - Information Sheets and Consent Forms

Refer to enclosed CD-ROM disc

Appendix B - Data analyses for Chapters 4, 5, 6, 7 & 8 and Appendix D

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Appendix C – Lenehan et al. (2015) *Psychogeriatrics* Reprint

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Appendix D – Lenehan et al. (2015) *Assessment* Reprint

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